RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

Federal Award Date: 03/06/2019

Grant Number: 1R01HL139673-01 REVISED
FAIN: R01HL139673

Principal Investigator(s):
Michael Hindle, PHD
P. Worth Longest (contact), PHD

Project Title: High Efficiency Delivery of Surfactant Aerosols to Infants without Intubation

Andrea Publow
Virginia Commonwealth University
Dir, OSP - Gov
800 East Leigh St, Suite 3200
PO Box 980568
Richmond, VA 232980568

Award e-mailed to: ospaward@vcu.edu

Period Of Performance:
Budget Period: 02/01/2018 – 01/31/2019
Project Period: 02/01/2018 – 01/31/2022

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to VIRGINIA COMMONWEALTH UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Heart, Lung, And Blood Institute of the National Institutes of Health under Award Number R01HL139673. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Ronald Caulder
Grants Management Officer
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

Additional information follows

SECTION I – AWARD DATA – 1R01HL139673-01 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$286,481
Fringe Benefits	$34,872
Personnel Costs (Subtotal)	$321,353
Materials & Supplies	$79,000
Travel	$9,000
Other	$52,566
Federal Direct Costs	$461,919
Federal F&A Costs	$230,534
Approved Budget	$692,453
Total Amount of Federal Funds Obligated (Federal Share)	$692,453
TOTAL FEDERAL AWARD AMOUNT	$692,453
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$692,453	$692,453
2	$688,568	$688,568
3	$723,687	$723,687
4	$734,852	$734,852

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Lung Diseases Research
CFDA Number:	93.838
EIN:	1546001758A1
Document Number:	RHL139673A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2018

IC	CAN	2018	2019	2020	2021
HL	8475150	$692,453	$688,568	$723,687	$734,852

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: LLLB N / **OC**: 414A / **Released**: eRA Commons User Name 03/04/2019
Award Processed: 03/06/2019 12:01:09 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01HL139673-01 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01HL139673-01 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants

Policy Statement, including addenda in effect as of the beginning date of the budget period.

e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01HL139673. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

REVISION # 2 - REDUCTION IN EFFORT FOR PI
The purpose of this revision is to reduce the PI Dr. Longest Academic Effort from [EFFORT] and Summer Effort from [EFFORT], in accordance with the letter dated Janurary 5th, 2018.

All other terms and conditions remain in effect.

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REVISION # 1- CORRECTION OF BUDGET/PROJECT END DATE(S)
The purpose of this revision is to correct both the budget/project end date(s) from 12/31/19 to 01/31/19. This notice of award supersedes the previous NOA issed on 01/24/2018. All terms and conditions listed below are still in effect.

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SPECIAL FUNDING GUIDELINE NOTICE
NIH is currently operating under a Continuing Resolution (See NIH Guide Notice NOT-OD-17-124: https://grants.nih.gov/grants/guide/notice-files/NOT-OD-17-124.html) and this award is being issued in accordance with the NHLBI FY 2018 Operating Guidelines which can be found at: https://www.nhlbi.nih.gov/research/funding/general/current-operating-guidelines.

GRADUATE STUDENT COMPENSATION
In accordance with the Notice: NOT-OD-02-017 entitled, GRADUATE STUDENT COMPENSATION published on December 10, 2001, in the NIH Guide for Grants and Contracts, total direct costs (salary, fringe benefits and tuition remission) for graduate students are provided at a level not to exceed the NIH maximum allowable amount (zero level of the Ruth L. Kirschstein National Research Service Award stipend in effect at the time of the competing award). Support recommended for future years has been adjusted accordingly, if applicable. The full guide Notice describing the level of compensation allowed for a graduate student can be found at: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-017.html

MULTIPLE PI - Prior Approvals
In keeping with NOT-OD-06-054 (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-06-054.html), as this award has multiple Principal Investigators (PIs), although the signatures of all the PIs are not required on prior approval requests submitted to the agency, the recipient institution must secure and retain the signatures of all of the PIs for their own internal processes.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Ronald Caulder
Email: caulderr@nhlbi.nih.gov **Phone**: 301.435.0148 **Fax**: 301-451-5462

Program Official: Aruna R. Natarajan
Email: aruna.natarajan@nih.gov **Phone**: 301-435-0222

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01HL139673-01 REVISED

INSTITUTION: VIRGINIA COMMONWEALTH UNIVERSITY

Budget	Year 1	Year 2	Year 3	Year 4
Salaries and Wages	$286,481	$286,481	$305,046	$305,046
Fringe Benefits	$34,872	$34,872	$53,222	$53,222
Personnel Costs (Subtotal)	$321,353	$321,353	$358,268	$358,268
Materials & Supplies	$79,000	$72,500	$69,750	$75,250
Travel	$9,000	$9,000	$6,000	$6,000
Other	$52,566	$54,668	$42,271	$43,963
Publication Costs		$1,500		
TOTAL FEDERAL DC	$461,919	$459,021	$476,289	$483,481
TOTAL FEDERAL F&A	$230,534	$229,547	$247,398	$251,371
TOTAL COST	$692,453	$688,568	$723,687	$734,852

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4
F&A Cost Rate 1	55%	55%	55.25%	55.25%
F&A Cost Base 1	$419,153	$173,440	$447,779	$454,971
F&A Costs 1	$230,534	$95,392	$247,398	$251,371
F&A Cost Rate 2		55.25%		
F&A Cost Base 2		$242,815		
F&A Costs 2		$134,155		

PI: **Longest, P. Worth**	Title: High Efficiency Delivery of Surfactant Aerosols to Infants without Intubation	
Received: 02/02/2017	FOA: PA16-160	Council: 10/2017
Competition ID: FORMS-D	FOA Title: NIH Research Project Grant (Parent R01)	
1 R01 HL139673-01	Dual: HD	Accession Number: 4013116
IPF: 353201	Organization: VIRGINIA COMMONWEALTH UNIVERSITY	
Former Number:	Department:	
IRG/SRG: GDD	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 461,919 Year 2: 464,531 Year 3: 485,466 Year 4: 497,185	Animals: Y Humans: Y Clinical Trial: N Current HS Code: Evaluati HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Michael Hindle	Virginia Commonwealth University	MPI
Philip Longest	Virginia Commonwealth University	PD/PI
Bruce Rubin	Virginia Commonwealth University	Co-Investigator
Rebecca Heise	Virginia Commonwealth University	Co-Investigator
Douglas Willson	Virginia Commonwealth University	Co-Investigator
Kelley Dodson	Virginia Commonwealth University	Co-Investigator

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

○ Pre-application ○ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED

Application Identifier FP00005536

c. Previous Grants.gov Tracking Number GRANT12328341

5. APPLICANT INFORMATION

Organizational DUNS*: 1053004460000

Legal Name*: Virginia Commonwealth University
Department: Mechanical Engineering
Division:
Street1*: 800 East Leigh St, Suite 3200
Street2: PO Box 980568
City*: Richmond
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 232980568

Person to be contacted on matters involving this application

Prefix: First Name*: Andrea Middle Name: J Last Name*: Publow Suffix:

Position/Title: Dir, OSP - Gov
Street1*: 800 East Leigh St, Suite 3200
Street2: PO Box 980568
City*: Richmond
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 232980568
Phone Number*: 8048286772 Fax Number: 8048282521 Email: dirospa@vcu.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1546001758A1

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ○ Resubmission
○ Renewal ○ Continuation ○ Revision

If Revision, mark appropriate box(es).

○ A. Increase Award ○ B. Decrease Award ○ C. Increase Duration
○ D. Decrease Duration ○ E. Other *(specify)* :

Is this application being submitted to other agencies?* ○Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
High Efficiency Delivery of Surfactant Aerosols to Infants without Intubation

12. PROPOSED PROJECT

Start Date* 09/01/2017
Ending Date* 08/31/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
VA-003

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Philip Middle Name: W Last Name*: Longest Suffix:
Position/Title: Professor
Organization Name*: Virginia Commonwealth University
Department:
Division:
Street1*: PO Box 843015
Street2:
City*: Richmond
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 232843015
Phone Number*: 8048277023 Fax Number: Email*: pwlongest@vcu.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$2,875,587.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$2,875,587.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Andrea Middle Name: J Last Name*: Publow Suffix:
Position/Title*: Dir, OSP - Gov
Organization Name*: Virginia Commonwealth University
Department:
Division:
Street1*: 800 East Leigh St, Suite 3200
Street2: PO Box 980568
City*: Richmond
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 232980568
Phone Number*: 8048286772 Fax Number: 8048282521 Email*: dirospa@vcu.edu

Signature of Authorized Representative*
Andrea.Publow

Date Signed*
02/02/2017

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter_v2.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Virginia Commonwealth University
Duns Number:	1053004460000
Street1*:	800 East Leigh St, Suite 3200
Street2:	PO Box 980568
City*:	Richmond
County:	
State*:	VA: Virginia
Province:	
Country*:	USA: UNITED STATES
Zip/ Postal Code*:	232980568
Project/Performance Site Congressional District*:	VA-003

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ○ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ● Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 ✓ 4 _ 5 _ 6

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number FWA0005287

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A3281-01

3. Is proprietary/privileged information included in the application?* ● **Yes** ○ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_Summary.pdf
8. Project Narrative*	Narrative.pdf
9. Bibliography & References Cited	References.pdf
10.Facilities & Other Resources	Facilities_and_Resources_surf_A0_v1.pdf
11.Equipment	Major_Equipment.pdf

Surfactant replacement therapy in neonates is currently achieved through endotracheal intubation and liquid bolus instillation. High efficiency delivery of aerosolized surfactant is proposed as a technique to improve airway distribution of the surfactant and prevent endotracheal intubation in cases where noninvasive ventilation (NIV) is the preferred respiratory support strategy. Primary limitations of aerosolized surfactants are currently very low lung delivery efficiencies (typically ~1%), long delivery times (~3 hours for mesh nebulizers), and poor distribution of the surfactant to the alveolar region.

The **goal** of this study is to develop formulations and devices for the effective delivery of aerosolized surfactants to the lungs of infants using the nose-to-lung (N2L) route thereby avoiding intubation. To achieve high efficiency lung delivery, the excipient enhanced growth (EEG) approach will be used in which submicrometer particles are formed through spray drying and contain the surfactant and a hygroscopic excipient. The initial small size of the aerosolized particles allows for effective penetration through the new delivery device and infant upper airways. Inclusion of the hygroscopic excipient in the primary particles fosters aerosol size increase inside the airways and effective deposition in the alveolar region. This approach was successfully employed by our group to improve N2L aerosol delivery in adults. The aerosol will be generated using new EEG surfactant powder formulations together with new low-flow and low-volume dry powder inhalers, which are developed and optimized using a combination of computational fluid dynamics (CFD), rapid prototyping, and *in vitro* experiments. Functionality of the new surfactant aerosol will be assessed in surfactant depletion animal models and compared with liquid instillation. The following aims are proposed to develop this new therapeutic approach:

Specific Aim 1. Develop an excipient enhanced growth (EEG) formulation of a lung surfactant that can be efficiently aerosolized, increase in aerodynamic size within the airways, and maintain surfactant function.

Specific Aim 2. Develop and optimize a device for generating and administering surfactant aerosols to infants using the noninvasive nose-to-lung (N2L) route and achieving high efficiency lung delivery.

Specific Aim 3. Adapt the N2L aerosol delivery device and test EEG surfactant aerosol efficacy in an infant-size ferret model compared with surfactant instillation in terms of oxygenation, lung distribution and histology.

Outcomes and Impact. Successful delivery of aerosolized surfactant will avoid the side effects associated with instillation in already compromised infant airways. Efficient N2L delivery will allow for expanded use of NIV respiratory support techniques, thereby avoiding the greater risks associated with intubation and liquid bolus instillation. In addition to respiratory distress syndrome in infants, improved surfactant delivery to the alveolar region may also aid the treatment of other lung conditions such as pneumonia and viral bronchiolitis.

Delivering surfactant replacement therapy to infants as an aerosol avoids the risks associated with tracheal intubation in cases where noninvasive ventilation is the preferred method of support and avoids the potentially harmful side effects associated with liquid bolus surfactant instillation. In this project, dry powder formulations and devices are developed for the effective delivery of aerosolized surfactants to the lungs of infants using the nose-to-lung route and thereby avoiding both tracheal intubation and liquid instillation. Advantages of the formulation and device combination include high efficiency delivery of the aerosol to the alveolar region, rapid dose delivery, and a convenient dry powder platform.

Facilities and Other Resources

Virginia Commonwealth University is the Commonwealth of Virginia's largest university, enrolling the most diverse student body in Virginia, with more than 31,000 undergraduate, graduate and professional students and employing more than 18,000 faculty and staff. VCU is a Carnegie Doctoral/Research University - Extensive, receiving over $250M in externally funded awards, including approximately $90M in funding from NIH in the past 12 months. Thirty-Three of VCU's graduate and professional programs are ranked by U.S. News and World Report as among the best in the nation, with two ranked first in the country. The VCU Health System, which includes the Medical College of Virginia Hospitals, has 1000 inpatient beds and is ranked in the top 100 U.S. hospitals. The VCU Institute for Women's Health is one of eighteen facilities designated as a National Center for Excellence in Women's Health by the US DHHS. VCU is closely affiliated with the adjacent Virginia Biotechnology Research Park.

In Vitro Laboratories

Dr. Hindle's aerosol and formulation laboratories (Smith, 461, 415, 421 – each 600 square feet) include three main equipment areas for (i) aerosol generation and characterization, (ii) solid state characterization and (iii) quantitative drug analysis.

Aerosol generation equipment includes the Buchi Nano Spray Dryer, the Capillary Aerosol Generator, the Small Particle Aerosol Generator, together with a number of commercial nebulizers and inhalers. The laboratories are equipped with a number of cascade impactors, including the 10 and 8 stage MOUDI Cascade Impactor, the Andersen Cascade Impactor, the Electrical Low Pressure Impactor and the Next Generation Impactor, all of which are standard methodologies for aerodynamic particle sizing of aerosols. In addition, the laboratory is equipped with a series of nasal, mouth-throat (MT) and tracheobronchial (TB) models that have been designed and validated for *in vitro* aerosol testing. Aerosol particle concentration can be measured using a condensation particle counter. Two breath simulators are available for use, including the ASL 5000 (IngMar Medical), which is a fully automated adult and neonatal breath simulator, together with an in-house inhalation simulator with a computer control system, which is capable of simulating various inhalation flow rates and wave patterns. An adult / infant test lung (Michigan Instruments) is also available for use in mechanical ventilation applications. For controlled temperature and humidity studies, the labs are equipped with two controlled temperature and humidity cabinets.

Physical characterization equipment includes the Differential Scanning Calorimeter (DSC) and Thermal Gravimetric Analyzer (TGA), both operated by the Pyris 7 Software (Perkin Elmer). The Malvern Mastersizer and the Sympatec Helos Rodos are state of the art laser diffraction particle sizers available in the laboratory. Other characterization equipment available includes a Karl Fisher apparatus and optical microscope. Scanning electron microscopy will be performed using the core university facilities provided by the Dept. of Neurobiology & Anatomy Microscopy Facility, which is supported, in part, with funding from NIH-NINDS Center core grant (5P30NS047463). Helium pychnometry is performed offsite by Micrometrics Analytical Services. Other items include rotameters, mass flow meters, compressed air source, vacuum pumps, humidifiers (Vapotherm 2000i), water baths, dry baths, oven, refrigerators, computers, lab furniture, and various durables and consumables. The functionality of the surfactant formulations with respect to their ability to reduce surface tension will be assessed using the BP2 Bubble Pressure Tensiometer (Kruss).

Quantitative drug analysis together with structural confirmation can be performed in Dr. Hindle's labs equipped with three Waters 2695 HPLC systems with auto-samplers, column heaters, UV PDA detectors and a fluorescence detector, together with a single quadrapole ZMD mass spectrometer and a triple quadrapole Quattro Micro mass spectrometer. The Quattro micro LC-MS will be utilized for the quantitative analysis of DPPC and other phospholipid and protein (surfactant proteins B and C) components of the spray dried EEG formulations.

Dr. Longest also maintains a separate experimental aerosol dynamics research laboratory (600 square feet) in the Department of Mechanical and Nuclear Engineering at VCU (Engineering East, E3263). This lab is equipped with a 3D Systems Viper SLA rapid prototyper machine for constructing replica models of the respiratory airways and prototype devices. For conducting aerosol experiments, existing equipment in this lab includes an assortment of impactors (Andersen and MOUDI), vacuum pumps, filters, and a sonicator. The nano-MOUDI is available for sizing aerosols at a flow rate of 5 L/min, which is applicable for pediatric and infant applications. A modified Electrical Low Pressure Impactor (ELPI by Dekati) is maintained for measuring

both aerosol charge and size. Various external voltage supplies (e.g., HP Model 6641A) are also present. The lab has an aerosol electrometer (TSI Model 3068A), for determining the charge on monodisperse particles and droplets, and a condensation particle counter (TSI Model 3022) for determining aerosol concentrations. The laboratory contains a variety of aerosol generation systems. Other items include rotameters, mass flow meters, compressed air sources, respiratory humidifiers, and an analytical balance.

Dr. Bruce Rubin's research space (2071 square feet) includes a molecular laboratory, biophysical laboratory, animal surgical room, and research support offices for the lab manager and technicians on the 8th floor of Sanger Hall and 2nd floor of Hermes A. Kontos Medical Sciences Building. The laboratory studies the relationships among inflammatory cells and mediators, infection, mucus secretion, and quality of life; and the mechanisms causing squamous metaplasia and goblet cell hyperplasia in the airway. The lab develops and tests new therapies from cell and tissue culture to animal models, aerosol delivery systems and clinical trials. The lab has studied physical and transport properties of mucus and sputum for 3 decades.

The molecular lab equipment includes 48w X 31d Fume Hoods (2), -80 Freezers (2), -20 Freezers (3), Chemical refrigerators (3), Millipore Direct Q3 Water system, Mettler Toledo Precision Balances (2), New classic MF and AE100, CKX41 Olympus Microscope, Zeiss AxioCam ICc-1 camera, BioTek ELx808 Absorbance Microplate Reader with incubator, Hot water bath,Thermo Max Q2000 shaker, VWR Incubating Micro-plat Shaker, Hotplate with Stirrer (3), Beckman Coulter Allegra 6 Series Centrifuge, Eppendorf Centrifuge 5417 C/R, Thermo scientific 1300 Series A2, Class Type A2 Biological Safety Cabinet, Nuaire, Class II Type A/B3, Biological Safety Cabinet, Bio-Rad CFX96 Time PCR Detection System, Bio-Rad Trans-Blot Turbo Blotting System, Bio-Rad TC10 Automated Cell Counter, Thermo Max Q2000 shaker, SensION pH 3 pH meter, Liquid Nitrogen Storage Dewar, Carbon Dioxide Tanks & Regulators.

The lab currently has four Dell OptiPlex 380 workstations, running Windows 7 equipped with JMP, Microsoft office 2010 for data acquisition and data processing. Select computers are equipped with Carl Zeiss Imaging Systems, GEN5, TA advantage, Photoshop and CFX Manager Software. All systems are equipped with RedCap Data Management.

The biophysical and animal room of the Rubin Lab is equipped with Dissection Microscopes Zeiss Stemi 2000-C and Nikon SMZ-2T, balances (2) Mettler Toledo AE100 and OHAUS ES Series, Labconco Freezone 4.5 Liter Benchtop Freeze Dry System, Contact Angle Imaging, TA Instruments AR1500ex Rheometer, Fisher Scientific Surface Tensiomat Model 21,Hot water/Shaker Bath (2) Bellco and Precision microprocessor controlled 280 series, Hotplate with Stirrer (1) and Cough Clearance Machine.

Lung Mechanics Laboratory

Dr. Rebecca Heise (Co-I) maintains 1500 sq ft of dedicated space in the School of Engineering Biotech One building at Virginia Commonwealth University in rooms 1076 and 1073. The lab contains all standard equipment necessary for molecular biology, tissue culture (including dedicated biosafety cabinet and incubators), and microscopy (inverted fluorescence microscope with imaging capabilities). Dr. Heise's animal work takes place in a dedicated barrier facility in the Moleular Medicine Research Building. Dr. Heise also has bench space within the VCU Victoria Johnson Research Center located on the 6th floor of the Molecular Medicine Research Building. This lung disease research laboratory occupies near 3,000 ft^2 of work space, structured with small laboratories designated for cell culture, animal experiments, RNA isolation, and high resolution microscopes, alongside 8 open laboratory benches for experiments. These resources will be utilized in the present study to assess efficacy of surfactant delivery in the surfactant depletion models.

Animal

VCU has an animal research program registered with the USDA (Customer and Certificate Numbers: 494 and 52-R-0007, respectively), assured via the NIH/PHS (Assurance Number: A3281-01), and fully accredited with the AAALAC (File Number: 00036). Animals for the proposed study will be housed in the Molecular Medicine Research Building. With an access limited only to the registered investigators, the facility is well maintained by dedicated personnel of the VCU Division of Animal Resources and veterinary staff. The rooms are tightly controlled with respect to temperature (20-23 °C), relative humidity (40-70 %) and light-dark cycling (12-12 h; the light cycle occurs between 6 am and 6 pm). Animals will be housed, fed and monitored under the appropriate practice compliance to the USDA, NIH/PHS and AAALAC regulations. The proposed studies will

be carried out under the protocol approved by the VCU Institutional Animal Care and Use Committee (IACUC). The compliance to the protocol will be ensured with the annual protocol review and semi-annual laboratory inspections by the VCU-IACUC. Both Dr. Rubin and Dr. Heise (who are responsible for conducting the animal experiments) have well maintained animal protocols.

Computer

Dr. Longest maintains a computational respiratory and aerosol dynamics lab (600 square feet) in the Department of Mechanical and Nuclear Engineering at VCU located in the new School of Engineering East Building (E3235). Presently, this lab features 8 dual processor workstations (2.8+GHz processors and 24+ GB of RAM in each machine). This lab maintains licenses to commercial CFD and FEA software, which are largely supplemented with a set of in-house user routines for mesh generation, particle dynamics, and pre/post-processing of results. Other available software includes Mimics, MatLab, MathCAD, Tecplot, Ensight, etc. Additional supercomputer resources are described below.

Supercomputing access is readily available to Dr. Longest through two on-campus VCU resources, which are the supercomputing Academic Technology Center and the Center for the Study of Biological Complexities (CSBC). The local lab interacts with these resources through high-speed internet connections. Resources readily available to these centers can be viewed at http://www.ts.vcu.edu/research/avail_resources.html#ts and include:

- Bach.vcu.edu – Linux Beowulf cluster – Computationally intensive applications
  - 500 processors, 2.6 GHz Opteron
  - 1 TB RAM (4GB per node)
  - 16.8 TB internal disk storage (73GB per node)

The VCU Academic Technology and CSBC supercomputing environment now has the following hardware totals: 800 parallel processors in Beowulf Clusters, 130 processors of SMP machines, 20 TB central storage (including nodes on the Beowulf, Fibre Channel, etc.). An additional 250 processors are being added. The Cluster is networked to the University Backbone with GigaBit Ethernet. In addition, the CSBC has a useful Advanced Scientific Visualization Laboratory.

As a faculty member, Dr. Longest and his research group have access to the computer resources at the Academic Technology Center. Dr. Longest is a member of the Center for the Study of Biological Complexity and has full access the computational resources managed by this center as well.

Office

Dr. Longest's office is located in the new Engineering East Building (Room 3250) of the VCU Academic Campus. This private office contains standard PCs with appropriate peripheral devices and software, a telephone, and a high-speed internet connection. Dr. Longest's office is on the same floor as his experimental and computational research labs. Dr. Longest maintains adequate separate office space to support the graduate student and postdoc that he will supervise in this project.

Dr. Hindle's office is located in the Smith Building (Room 442) on the VCU Medical Campus. His private office contains a standard PC with appropriate peripheral devices and software, a telephone, and a high-speed internet connection. Dr. Hindle's office is in the same building as his experimental research lab. Dr. Hindle has available laboratory office space sufficient for the proposed personnel in this project; a total of 3 office desk spaces are available in Smith Rooms 461 and 415. The laboratories are equipped with desktop computers with appropriate peripheral devices and software, telephones, and high-speed internet connections.

Dr. Heise's office is located in VCU BioTech One adjacent to her research laboratories and the VCU Medical Campus. Her private office space is quite sufficient to conduct her duties associated with this project, and she has adequate separate office space in BioTech One to support the student she will supervise.

Dr. Rubin, Dr. Willson and Dr. Dodson each maintain academic offices located on the VCU Medical Campus, which are adequately equipped to conduct their components of this project.

These office spaces are quite sufficient to support the proposed research. All offices are located within approximately two miles of each other. This proximity on the VCU campus will allow for frequent meetings between the experimental and computational research groups to report progress and to compare findings, as well as frequent meetings with clinical collaborators.

Scientific Environment

Dr. Longest (PI; multiple PI model) holds joint appointments in the Department of Mechanical and Nuclear Engineering and the Department of Pharmaceutics at VCU. Dr. Hindle (PI; multiple PI model) holds a faculty appointment in the Department of Pharmaceutics, and Dr. Heise holds a faculty appointment in the Department of Biomedical Engineering. Dr. Rubin is the Jessie Ball DuPont Distinguished Professor and Chair of the VCU Department of Pediatrics and the Physician-in-chief of Children's Hospital of Richmond at VCU. Dr. Willson is Professor and Chief of Pediatric Critical Care Medicine at VCU and Dr. Dodson is an Associate Professor of Otolaryngology/Head and Neck Surgery at VCU. The VCU Department of Pharmaceutics is home to the internationally recognized Aerosol Research Group and is responsible for organizing the annual and well-known Respiratory Drug Delivery (RDD) conference series. This conference currently draws approximately 750 participants from academia and industry to national and international conference sites each year. The Aerosol Research Group at VCU has made major contributions to the field of respiratory drug delivery for over 20 years. The group holds weekly discussion group meetings to exchange their research ideas and outcomes, and experimental collaborations and assistances between their laboratory personnel are routine.

While at VCU, Drs. Longest and Hindle have fostered an effective collaboration in the field of respiratory drug delivery. This collaboration brings together Dr. Longest's expertise in transport phenomena and computational fluid dynamics modeling with Dr. Hindle's expertise in inhaled pharmaceutical aerosols, *in vitro* experiments, and pharmaceutical chemistry. The *in vitro* component of this collaboration provides physically realistic results of aerosol transport and deposition in medical inhaler devices and anatomical models of the respiratory tract. The CFD modeling is validated based on the *in vitro* deposition results and provides significant details related to the underlying transport mechanisms and local deposition characteristics. Using this approach, they have successfully completed one industrial sponsored project on inhaler development, one contract and one U01 award from the US FDA on inhaler modeling and *in vitro* airway geometry development, and three R21 grants from NHLBI and NICHD on respiratory drug delivery techniques. Currently, Dr. Hindle and Dr. Longest collaborate on one R01 project (in NCE) dealing with controlled condensational growth during non-invasive ventilation in adults and a US FDA project (also in NCE) on predicting *in vivo* aerosol deposition. These awards have produced over twenty journal papers in a three-year period, of which fourteen publications were supported by NIH grants. These previous and existing collaborative projects have resulted in a regular dialogue between Dr. Longest and Dr. Hindle, and their respective labs.

Dr. Willson has worked with surfactant replacement therapy in critical care medicine for over a decade. He has published several seminal papers on the subject and led large-scale clinical trials on the use of surfactant replacement therapy. In this project, Dr. Willson will provide clinical insights in the delivery of surfactant replacement therapy to infants and advise the PIs on practical aspects of implementing the newly developed delivery devices in patients. Dr. Longest and Dr. Dodson currently collaborate on an existing R01 award and participate together in a Nasal Interest Research Group at VCU. This group consists of clinical researchers, biomedical engineers, and biological scientists with a focus on nasal applications, such as non-invasive ventilation and nasal drug delivery for adults, children and infants. Dr. Dodson will assist with identifying the required nasal and airway CT scans and will ensure that the selected scans do not contain nasal abnormalities. Through collaboration with an otolaryngologist and clinical expert in surfactant delivery, the PIs intend to develop practical and realistic aerosol delivery strategies and devices for neonates.

Dr. Rubin brings invaluable experience to the team in the areas of clinical administration of surfactant aerosols to children and aerosol delivery to an infant-size ferret animal model. As Physician-in-chief of Children's Hospital of Richmond at VCU, Dr. Rubin together with Dr. Willson will provide invaluable clinical insight in the development of a practical delivery device. Dr. Rubin has conducted research on surfactant replacement therapy for over 25 years. Furthermore, Dr. Rubin has over 25 years of experience with ferret animal models, which are the correct size to represent human infant airways. Dr. Rubin and Dr. Longest have previously completed one funded industrial project [Private Source] together, which resulted in multiple journal articles, and have co-authored several additional conference abstracts. In future stages of this project (beyond the current grant proposal), continued collaborations with these and other clinicians are envisioned in order to test the aerosol delivery systems and new aerosol surfactant replacement therapy in infants at VCU.

This project will enable a new collaboration between the PIs and Dr. Rebecca Heise, an expert in lung injury, inflammatory pulmonary markers, and animal lung models. This new collaboration will test the *in vivo* efficacy of the deposited surfactant aerosol particles in an animal model of surfactant depletion. This will

ensure that the spray dried surfactant formulation retains its function after delivery and deposition *in vivo*. Moreover, Dr. Heise's extensive experience in airway inflammatory markers will provide valuable insight into potential benefits of surfactant aerosol delivery compared with liquid bolus instillation. Continued evidence that aerosol delivery produces less pulmonary inflammation compared with bolus instillation will potentially lead to its adoption during non-invasive and invasive mechanical ventilation. Moreover, this new collaboration will test excipient enhanced growth (EEG) aerosol performance in an animal model, for the first time, and will lead to future interactions evaluating the effectiveness of other pulmonary therapies developed by Dr. Hindle and Dr. Longest with *in vivo* animal lung injury models.

Major Equipment

Nano Spray Dryer – Department of Pharmaceutics. This next generation Buchi spray dryer incorporates a novel vibrating mesh mechanism and electrostatic precipitator to generate spray dried particles in the size range 0.3µm - 10µm. It can operate in open or closed loop modes for aqueous and organic solvents, respectively. This spray dryer will be used to generate submicrometer combination particles for surfactant formulations. This equipment has been successfully used to generate submicrometer combination particles for drugs with differing physico-chemical properties including albuterol sulfate, budesonide, ciprofloxacin and insulin. We have successfully employed the spray dryer to produce submicrometer Survanta-mannitol and Survanta-sodium chloride EEG combination particles during our preliminary studies.

Breath Simulators – Department of Pharmaceutics. Two breath simulators are available. The first system is the ASL 5000 (Ingmar Medical), which is a digitally controlled, high fidelity breathing simulator. It has a precision flow waveform generator suitable for aerosol delivery device testing in both adults and children. The 2nd breath simulator was developed in-house and enables an actual inhalation flow profile to be recorded using PC-based data acquisition software. Simulated profiles can also be made using the in-house software. The transient flow rate profiles are then able to be replayed using an independent airflow source suitable for *in vitro* evaluation of the noninvasive ventilation delivery systems.

Adult/Infant Test Lungs – Department of Pharmaceutics. Two test lung systems are available, the Dual Adult Lung and the Adult/Infant Lung (Michigan Instruments). These are designed to simulate a wide variety of pulmonary conditions. Lung compliance and resistances can be adjusted to simulate any type of pathology in either adult or infant ventilation scenarios. This device offers a wide range of compliance and resistance settings from 5-500 cm H_2O/L/sec (resistance) and 0.001-0.10 L/cmH_2O (compliance).

Cascade Impactors – Department of Pharmaceutics. Andersen Cascade impactor, Moudi 8 and 10 stage impactors, Electrical Low Pressure Impactor, Next Generation Impactor and Nano-Moudi. The Aerosol Research Group is equipped with a number of pharmaceutical impactors capable of fractionating and sizing aerosols over a large particle size range from 50 nm to 10 µm. An appropriate impactor will be employed to determine the particle size distribution of aerosols generated by the prototype systems and novel formulations and measure any change in size due to condensational growth. The Nano-Moudi is appropriate for low flow studies as will be necessary for neonatal applications.

High performance liquid chromatography with photodiode array or fluorescence detector or single and triple quadrapole mass spectrometer (HPLC and LC-MS) – Department of Pharmaceutics. Alliance 2695 (Waters Corp). This analytical equipment will be dedicated to the drug quantification analysis and mass spectral identification required throughout this project encompassing the pharmaceutical characterization, stability studies, aerodynamic particle sizing, and *in vitro* deposition testing. LC-MSMS separation and detection (Quattro Micro, Waters Corp) will be used for analysis of these complex surfactant formulations.

Differential Scanning Calorimeter (DSC) and Thermogravimetric Analyzer (TGA) – Department of Pharmaceutics. The DSC7 and Pyris 1 TGA (Perkin Elmer Corp) are characterization methods that will be used to evaluate the submicrometer EEG particles of surfactant and hygroscopic excipients.

Bubble Pressure Tensiometer – Department of Pharmaceutics. The KP2 (Kruss) bubble pressure tensiometer will be employed to investigate the functionality of the generated surfactant EEG powders to measure dynamic and equilibrium surface tension.

Laser Diffraction Particle Sizer for rapid screening – Department of Pharmaceutics. State funding has been provided for the Department of Pharmaceutics to purchase a new state of the art laser diffraction particle sizer. The HELOS RODOS system (Sympatec) will be equipped with the ASPIROS accessory for sizing milligram quantities of powders for rapid screening of spray dried products.

Scanning Electron Microscopy (SEM). We have access to Scanning Electron Microscopy (Zeiss EVO 50 XVP) facilities in the VCU - Dept. of Anatomy & Neurobiology Microscopy Facility, which is supported with funding from NIH-NINDS Center core grant 5P30NS047463, NIH-NCI Cancer Center Support Grant P30 CA016059 and NIH-NCRR grant 1S10RR022495.

SLA Rapid Prototyper – Department of Mechanical Engineering. 3D Systems Viper SLA (stereolithography) machine. This rapid prototyping system uses a 100 mW solid-state laser to selectively harden Accura 60 (3D Systems) plastic resin into a CAD-based geometry. The in-house rapid prototyper will be used to construct hollow *in vitro* models of the respiratory tract and prototypes of the delivery devices.

PolyJet Rapid Prototyper – Department of Mechanical Engineering. Objet24 and Objet Eden260VS 3D Printers. These 3D printers have a build layer thickness of 30 µm providing extremely high resolution reconstruction of CAD-based geometries. These in-house 3D printers will be used to construct hollow *in vitro* models of the respiratory tract.

In Vitro Nose-MT-TB Deposition Model – Departments of Mechanical Engineering and Pharmaceutics. Characteristic models of the nose, mouth-throat (MT) and upper tracheobronchial (TB) airways have been developed by the VCU Department of Mechanical Engineering and used for medical aerosol testing in the VCU Department of Pharmaceutics. These geometries have demonstrated good agreement with *in vivo* aerosol deposition studies in terms of both extrathoracic deposition and lung delivery. Currently, these Nose-MT-TB geometries have been employed to test over 12 aerosol delivery platforms in published studies with excellent agreement to *in vivo* results. In the current study, a new neonatal nose-MT-TB deposition model will be developed for infants receiving noninvasive ventilation.

SciReq flexiVent FX lung mechanics measurement system with rat module – Department of Biomedical Engineering. The flexiVent FX can be used to obtain resistance and compliance measurements, forced oscillation techniques, partitioned respiratory mechanics, parallel FOT, pressure-volume loops, and lung volumes. The flexiVent will be utilized to mechanically ventilate rats in the present study following lavage injury and surfactant delivery. Measurements obtained will include resistance, compliance, and elastance measurements. Pressure volume loops will also be obtained as measures of lung injury.

Siemans RL248 Blood Gas Machine – Department of Biomedical Engineering. The 248pH Blood Gas System will be used for the determination of pH, both the concentration of carbon dioxide [pCO2] and the concentration of oxygen [pO2] in heparinized whole blood samples. In addition to the measured parameters, the 238pH Blood Gas System will also calculate standard or actual bicarbonate (HCO3) and total carbon dioxide (tCO2) base excess in vivo or in vitro (BEvv or BEvt) and oxygen saturation (O2 sat). Features of the Siemens 238pH Blood Gas System include results that are displayed within 20 to 120 seconds of returning the probe with 60uL (capillary) or 85uL (syringe) nominal sample volumes and automatic calibration programmable sequence. In the present study the blood gas machine will be utilized to obtain information on the oxygenation levels of the surfactant depleted rats +/- surfactant therapy.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

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Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 2002

Attach Biographical Sketch*: File Name: Longest_biosketch.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-16-160 . Received Date: 2017-02-02T16:07:33.000-05:00

PROFILE - Senior/Key Person

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Degree Type: PhD Degree Year: 1992

Attach Biographical Sketch*: File Name: Hindle_biosketch.pdf

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Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Rubin_biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Heise_biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Willson_biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Dodson_biosketch.pdf

Attach Current & Pending Support: File Name:

OMB No. 09250001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Longest, Philip Worth

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Professor of Mechanical and Nuclear Engineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
North Carolina State University, Raleigh, NC	B.S.	12/1996	Mechanical Engineering
North Carolina State University, Raleigh, NC	M.S.	12/1999	Mechanical Engineering
North Carolina State University, Raleigh, NC	Ph.D.	10/2002	Mechanical Engineering
North Carolina State University, Raleigh, NC	Postdoc	6/2003	Multiphase Transport
U.S. Environmental Protection Agency, RTP, NC	Postdoc	8/2004	Respiratory Dosimetry

A. Personal Statement

My graduate training was in the areas of multiphase transport and computational modeling. I completed postdoctoral projects at both NC State and the U.S. Environmental Protection Agency (EPA), National Exposure Research Lab, on aerosol physics and the respiratory dosimetry of inhaled gases and particles. I joined the VCU Engineering faculty as an assistant professor in 2004, received a joint appointment to the Department of Pharmaceutics in 2006, and currently hold a full professor position. My current research at VCU centers on (i) developing effective new methods for generating and delivering medical aerosols and (ii) developing numerical models and airway geometries to assess and improve respiratory drug delivery. As an individual and in collaboration with the VCU Department of Pharmaceutics, I have secured funding from corporate and federal (NIH, NSF, US FDA) sponsors. In each of these projects, I have overseen successful collaborations and effective output in terms of conference presentations, peer-reviewed publications, and intellectual property generation. Over the last five years, my research group has published over 45 peer-reviewed papers with over 30 of these in collaboration with the VCU Department of Pharmaceutics.

For the proposed project, Dr. Michael Hindle and I will serve as multiple PIs. Dr. Hindle brings expertise in the areas of inhaler development and design, *in vitro* aerosol experiments, and pharmaceutical chemistry. This compliments my strengths in multiphase transport, medical devices, and numerical modeling including computational fluid dynamics (CFD).

The proposed study is a logical extension of my collaborative work with Dr. Hindle on improved techniques for effective respiratory drug delivery. Dr. Hindle and I received an NIH R21 award, which is now complete, to develop the excipient enhanced growth (EEG) concept for orally inhaled drug products. In this project, we showed that the EEG approach could nearly eliminate mouth-throat depositional loss and produce aerosol size increase sufficient for full lung retention using both spray and dry powder devices. Our approach of combining experiments and CFD modeling helped to rapidly develop this technique for multiple inhaler platforms. Dr. Hindle and I currently collaborate on an active NIH R01 award that implements condensational growth technology to deliver medical aerosols during non-invasive ventilation (NIV) in adults. The NIV approach involves improving the delivery of nebulized aerosols through ventilator tubing, nasal interfaces, and the nasal cavity for effective lung delivery of liquid-based products. In contrast, the proposed project deals with spray dried surfactant powders delivered to infants receiving NIV. Dr. Hindle and I also collaborated on two US Food and Drug Administration (FDA) sponsored projects with the goal of developing combination *in vitro* and CFD models for testing new inhalers and pharmaceutical products. These FDA funded projects demonstrate the benefit of using a concurrent *in vitro* and CFD

approach for rapid and effective device and delivery strategy optimization. It also highlights the interest of the US FDA in using this type of concurrent approach for product development and assessment of *in vivo* performance. Recently, Dr. Hindle and I completed an NIH R21 project on the delivery of nebulized aerosols to infants receiving invasive mechanical ventilation. Results of this project highlight optimal particle size for improving lung penetration and minimizing aerosol exhalation together with targeted lung delivery using either EEG or aerosol charge.

The objective of this proposed study is to develop formulations and devices for the effective delivery of aerosolized surfactant to the lungs of infants receiving NIV. Advantages of the formulation and device combination include high efficiency delivery of the aerosol to the alveolar region, rapid dose delivery, and a convenient DPI platform. Clinically, delivering the aerosol during NIV will avoid the risks associated with invasive mechanical ventilation when it is performed only for surfactant administration and the potentially dangerous side effects of liquid bolus instillation. The delivery approach is based on controlled condensational growth technology, which was initially developed by my research team in collaboration with Dr. Hindle. Additional concepts that are included in this proposal and were developed by my group in collaboration with Dr. Hindle include high efficiency inline 3D rod array DPIs and streamlined nasal cannula for efficient nose-to-lung aerosol delivery. These new concepts play a critical role in making efficient lung targeted delivery of surfactants possible in infants without the need to intubate.

1. Tian, G., Hindle, M., Lee, S., Longest, P. W. 2015. Validating CFD predictions of pharmaceutical aerosol deposition with in vivo data. *Pharmaceutical Research*. 32(10): 3170-3187. (PMID: 25944585)
2. Golshahi, L., Longest, P. W., Azimi, M., Syed, A., and Hindle, M. 2014. Intermittent aerosol delivery to the lungs during high flow nasal cannula therapy. *Respir Care* 59(10): 1476-1486. (PMID: 24917454)
3. Tian, G., Longest, P. W., Li, X., and Hindle, M. 2013. Targeting aerosol deposition to and within the lung airways using excipient enhanced growth. *Journal of Aerosol Medicine and Pulmonary Drug Delivery* 26(5): 248-265. (PMC3826577)
4. Longest, P. W. and Holbrook, L. T. 2012. In silico models of aerosol delivery to the respiratory tract – Development and applications. *Advanced Drug Delivery Reviews* 64: 296-311. (PMC3258464)

B. Positions and Honors

Positions and Employment

1997 – 2002	**Research Assistant** North Carolina State University, Department of Mechanical and Aerospace Engineering *Research focus*: Computational particle hemodynamics
2002 – 2003	**Post-doctoral Research Fellow** U.S. Air Force Office of Scientific Research *Research focus*: Biophysical particle transport
2003 – 2004	**Post-doctoral Research Fellow** U.S. Environmental Protection Agency, Exposure Modeling Research Branch *Research focus*: Respiratory airway dynamics of particulate matter
2004 – 2009	**Assistant Professor** of Mechanical Engineering Virginia Commonwealth University
2007 – 2009	**Qimonda Assistant Professor** of Mechanical Engineering Virginia Commonwealth University
2009 – 2012	**Associate Professor** of Mechanical Engineering Virginia Commonwealth University
2012 – Present	**Professor** of Mechanical and Nuclear Engineering; and Professor of Pharmaceutics Virginia Commonwealth University *Research focus*: Targeted drug delivery and pharmaceutical aerosols

Honors

- U. S. Environmental Protection Agency Complimentary Award, 1998
- NCSU ME graduate research awards 2001 and 2002
- North Carolina Supercomputing Center Cray/SGI Fellowship, 1999
- NSF Graduate Research Trainee Fellowship in Scientific Computation, 1998
- Graduate Assistance in Areas of National Need (GAANN) Fellowship, 2000

- Qimonda Associate Professor of Mechanical Engineering endowed professorship, 2007-2009
- American Institute of Medical and Biomedical Engineers, Fellow 2016

Memberships

International Society of Aerosols in Medicine (ISAM); American Association for Aerosol Research (AAAR); American Society of Mechanical Engineers (ASME); Biomedical Engineering Society (BMES); American Society for Engineering Education (ASEE); Phi Kappa Phi Honor Society

C. Contributions to Science

1. Targeted Drug Delivery to and Within the Lungs

Current pharmaceutical inhalers are generally inefficient at delivering inhaled medications to the lungs and do not effectively target deposition within the airways. In collaboration with Dr. Michael Hindle in the VCU Department of Pharmaceutics, my research seeks to develop optimized techniques and new delivery strategies to maximize the lung delivery efficiency of inhaled pharmaceuticals and to better target deposition within the lungs. One new technique I have developed in collaboration with Dr. Hindle is excipient enhanced growth (EEG), in which submicrometer particles are composed of an active pharmaceutical agent and hygroscopic excipient. Inclusion of the hygroscopic excipient causes the particle to take up water in the humid respiratory airways and deposit. By controlling the inhalation flow rate and amount of hygroscopic excipient, targeted deposition of the inhaled medication can be achieved. As a second example, my research explores the optimal selection of particle size and charge to target deposition within the airways of infants on mechanical ventilation. Considering EEG delivery, CFD and *in vitro* estimates indicate negligible mouth-throat depositional loss (~1% of the aerosolized dose in most cases), compared with 40-90% for conventional devices. Size increase of the particles or droplets composing the aerosol is observed to be significant resulting in full lung retention. Using a newly developed whole-lung CFD model, deposition in the currently underserved small tracheobronchial airways was increased by 40-fold compared with conventional inhalers. Negligible extrathoracic deposition has also allowed for the effective use of nose-to-lung delivery. This nasal interface approach is useful for administering medications during non-invasive ventilation (NIV) such as new high flow nasal cannula techniques or for delivering medications to children.

1. Tian, G., Hindle, M., Longest, P. W. 2014. Targeted lung delivery of nasally administered aerosols. *Aerosols Science and Technology* 48(4): 434-449. (PMC4051279)
2. Golshahi, L., Tian, G., Azimi, M., Son, Y.-J., Walenga, R., Longest, P. W., Hindle, M. 2013. The use of condensational growth methods for efficient drug delivery to the lungs during noninvasive ventilation high flow therapy. *Pharmaceutical Research*. 30:2917-2930. (PMC3800269)
3. Longest, P. W., Tian, G., Li, X., Son, Y.-J., Hindle, M. 2012. Performance of combination drug and hygroscopic excipient submicrometer particles from a softmist inhaler in a characteristic model of the airways. *Annals of Biomedical Engineering*. 40: 2596-2610. (PMC3504134)
4. Hindle, M. and Longest, P. W. 2012. Condensational growth of combination drug-excipient submicrometer particles for targeted high efficiency pulmonary delivery: Evaluation of formulation and delivery device. *Journal of Pharmacy and Pharmacology*. 64(9): 1254-1263. (PMC3419492)

2. CFD and In vitro Models of Pharmaceutical Aerosol Delivery

Computational fluid dynamics (CFD) provides a powerful tool for predicting the transport and deposition of gases and particles in the respiratory tract. However, due to the complexity of the respiratory airways, CFD models are typically limited to relatively small regions. At VCU, my group has developed the stochastic individual pathway (SIP) modeling approach to simulate whole-lung aerosol transport and deposition with CFD. The approach simulates the upper airways through approximately the lobar bronchi using characteristic models derived from CT scans. These models are also rapid prototyped for generating corresponding *in vitro* deposition data. Transport and deposition in the remainder of the tracheobronchial airways is then evaluated using the SIP approach in which ensembles of individual pathways are created and simulated. Finally, alveolar deposition is simulated using a new space-filling alveolar geometry that approximates a complete acinus with airflow driven by wall motion. We have extensively validated CFD predictions of upper airway deposition with concurrent *in vitro* data (from Dr. Hindle) for MDIs, DPIs, nebulizers, and softmist inhalers. The SIP approach was demonstrated as an effective method to simulate lung deposition of pharmaceutical aerosols with a

computational savings of multiple orders of magnitude compared with simulating all of the tracheobronchial airways. Implementation of the new alveolar model provides, for the first time, a mechanism to simulate complete airway deposition of pharmaceutical aerosols using CFD. Comparisons between CFD predictions and *in vivo* data of regional lung deposition are in very close agreement.

1. Longest, P. W., Tian, G., Khajeh-Hosseini-Dalasm, N., Hindle, M. 2016. Validating whole-airway CFD predictions of DPI aerosol deposition at multiple flow rates. *Journal of Aerosol Medicine and Pulmonary Drug Delivery* 2016; 29(3):461-481 doi: 10.1089/jamp.2015.1281. PMID: 27082824
2. Longest, P. W., Tian, G., Delvadia, R., Hindle, M. 2012. Development of a stochastic individual path (SIP) model for predicting the deposition of pharmaceutical aerosols: Effects of turbulence, polydisperse aerosol size, and evaluation of multiple lung lobes. *Aerosol Sci Tech* 46(12): 1271-1285.
3. Longest, P. W., Tian, G., Walenga, R. L., Hindle, M. 2012. Comparing MDI and DPI aerosol deposition using in vitro experiments and an new stochastic individual path (SIP) model of the conducting airways. *Pharmaceutical Research.* 29 1670-1688. (PMID22290350)
4. Delvadia, R., Longest, P. W., Byron, P. R. 2012. In vitro tests for aerosol deposition. I: Scaling a physical model of the upper airways to predict drug deposition variation in normal humans. *Journal of Aerosol Medicine and Pulmonary Drug Delivery.* 25(1) 32-40. (PMID22070526)

3. Development of Therapeutic Devices using a Combination of CFD, Rapid Prototyping and In vitro Testing

As described above, a submicrometer aerosol strategy can be used to minimize mouth-throat depositional loss. However, creating a submicrometer aerosol with a dry powder inhaler (DPI) is a considerable challenge. In collaboration with the VCU Department of Pharmaceutics, we have developed a formulation and DPI device combination capable of producing a submicrometer aerosol. The DPI device was based on CFD simulations, which indicated that a new 3D rod array structure could most effectively deaggregate the powder formulation The resulting DPI product was then optimized through a combination of CFD simulations, rapid prototyping, and *in vitro* testing. This new line of inhalers is capable of producing submicrometer aerosols with negligible mouth-throat depositional loss and little sensitivity to inhalation flow rate. Furthermore, inline versions of the DPI were developed for application to ventilation tubing and aerosol delivery during noninvasive ventilation or for the delivery of aerosols to children that are too young to use an inhaler. As a second example of contributions in this area, my group has employed CFD, rapid prototyping, and in vitro testing to develop new mechanical ventilation components and patient interfaces that dramatically improve (up to 10x) aerosol delivery to patients receiving either invasive or noninvasive ventilation.

1. Behara, S. R. B., Farkas, D. R., Hindle, M., Longest, P. W. 2014. Development of a high efficiency dry powder inhaler: Effects of capsule chamber design and inhaler surface modifications. *Pharmaceutical Research* 31:360-372. (PMC3946921)
2. Longest, P. W., Son, Y.-J., Holbrook, L., and Hindle, M. 2013. Aerodynamic factors responsible for the deaggregation of carrier-free drug powders to form micrometer and submicrometer aerosols. *Pharmaceutical Research* 30: 1608-1627. (PMC3703624)
3. Son, Y.-J., Longest, P. W., Tian, G., Hindle, M. 2013. Evaluation and modification of commercial dry powder inhalers for the aerosolization of a submicrometer excipient enhanced growth (EEG) formulation. *European Journal of Pharmaceutical Sciences* 49: 390-399. (PMC3744372)
4. Longest, P. W., Golshahi, L., Hindle, M. 2013. Improving pharmaceutical aerosol delivery during noninvasive ventilation: Effects of streamlined components. *Annals of Biomedical Engineering* 41: 1217-1232. (PMC3647043)

4. Delivering Pharmaceutical Aerosols to Infants Receiving Mechanical Ventilation

Aerosol delivery to infants receiving mechanical ventilation is very inefficient. As a result, it is often unclear if many inhaled medications are not effective in infants at the biological level or if a lack of efficacy is due to insufficient delivery efficiency and dose targeting within the lungs. In collaboration with Dr. Hindle, we have employed a combination of CFD simulations and *in vitro* tests to optimize aerosol delivery efficiency to infants receiving invasive mechanical ventilation. We developed streamlined ventilation components that significantly (5x) improve aerosol transmission. With static size aerosols, we identified optimal conditions that minimize both delivery system deposition and exhalation of the dose in order to maximize lung delivery.

Use of EEG further improves lung aerosol delivery in ventilated infants with very high (~70%) alveolar delivery efficiencies.

1. Longest, P. W. and Tian, G. 2015. Development of a new technique for the efficient delivery of aerosolized medications to infants on mechanical ventilation. *Pharmaceutical Research* 32: 321-336. (PMC4286504)
2. Longest, P. W., Mandana, A., and Hindle, M. 2014. Optimal delivery of aerosols to infants during mechanical ventilation. *Journal of Aerosol Medicine and Pulmonary Drug Delivery* 27(5): 371-385. (PMC4227441)

Complete List of Published Works: https://scholar.google.com/citations?user=w8MxYJYAAAAJ&hl=en

D. Research Support

Ongoing Research Support

Longest (PI), Hindle (PI), Mossi (I), and Dodson (I) 6/1/11 – 3/31/17
NIH/NHLBI R01
Title: Effective Delivery of Pharmaceutical Aerosols during Non-Invasive Ventilation
Objective: The objective of this study is to develop pharmaceutical delivery systems for adults that can significantly enhance aerosol deposition in the lungs during non-invasive ventilation (NIV) using a condensational growth approach.

Hindle (Co-PI), Longest (Co-PI) 9/30/13- 9/29/17
University of Florida (Subcontract from US FDA contract # HHSF223201310223C)
Title: Applicability of in silico, in vitro and pharmacokinetics studies to elucidate critical API changes in nasal suspension formulations.
Objective: The objective of this study is to develop in vitro and CFD methods to characterize nasal spray suspension products.

Completed Research Support

Longest (PI), Hindle (I), Byron (I) 9/15/12 – 9/14/16
US FDA U01
Title: Predictive Lung Deposition Models for Safety and Efficacy of Orally Inhaled Drugs
Objective: The objective of this study is to develop combination CFD and *in vitro* adult airway models that can predict the *in vivo* deposition of orally inhaled drugs across a population.

Longest (PI), Hindle (I) and Tepper 8/5/12 – 4/30/16
NIH/NHLBI R21
Title: Nanoaerosols from Wick Electrospray for Improved Drug Delivery to Infants
Objective: This project seeks to use charged nanoparticles to enhance respiratory drug delivery to mechanically ventilated infants.

Longest (PI) and Hindle (PI) 7/1/10 – 6/30/13
NIH/NHLBI R21
Title: Excipient Enhanced Aerosol Particle Formulations and Inhaler Development for Improved Pulmonary Drug Delivery
Objective: The overall goal of this project was to develop a novel technology for the efficient delivery of inhaled nanoparticles to adults that minimizes deposition in the mouth and throat while maximizing delivery to the lungs.

Rubin (PI) and Longest (I) 12/1/11 - 10/1/12
Private Source
Title: Construction of a Biomechanical Model to Study Intubation and Mucus Aspiration
Objective: This project constructed a flexible model of the upper airways to study intubation.

Longest (PI) and Hindle (I) 9/15/10 – 9/14/11
US FDA
Title: Computational Study of Lung Deposition for Orally Inhaled Drug Products
Objective: The objective of this study was to develop validated computational models for efficiently predicting the delivery of orally inhaled drug products.

OMB No. 09250001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Hindle, Michael

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Bradford, Bradford	OTH	06/1989	Pharmacy
University of Bradford, Bradford	PHD	12/1992	Pharmaceutical Sciences
Virginia Commonwealth University, Richmond	Postdoctoral Fellow	12/1994	Aerosol Science

A. Personal Statement

I have broad experience in a number of areas of inhalation drug delivery science encompassing human pharmacokinetic studies, dry powder inhaler formulation design, in vitro test methodology development, analytical method development and the investigation of novel aerosol generation techniques. As a direct result of my research efforts, I have been assigned 9 patents and was successful in incorporating this research into the training of 5 Ph.D. students and a number of post-doctoral scientists. My training as a pharmaceutical scientist was under the guidance of Professor Henry Chrystyn (Ph.D.) and Dr. Peter Byron (Post Doc Fellow), two leaders in the area of inhalation drug delivery. I am currently the Peter R. Byron Distinguished Professor at Virginia Commonwealth University where I lead a group of 2 graduate students and a Post Doc Fellow. I have built a successful collaboration with Dr. Worth Longest (Department of Mechanical Engineering, VCU) which has successfully obtained NIH, FDA and industrial funding.

For the proposed project, Dr. Longest and myself will serve as multiple PIs. Dr. Longest's skills in computational fluid dynamics, aerosol science, and engineering device design approached from an engineering perspective align well with my pharmaceutical science skill set. We have pioneered the use of CFD and *in vitro* aerosol drug deposition testing to establish *in silico – in vitro – in vivo* correlations for inhaled drug products in work sponsored by the US FDA, which resulted in a number of publications. These *in silico – in vitro* methods will be employed to characterize the devices and delivery methods that will be developed in this project, prior to future clinical testing. These FDA funded projects demonstrate the benefit of using a concurrent *in vitro* and CFD approach for rapid and effective device and delivery strategy optimization. It also highlights the interest of the US FDA in using this type of concurrent approach for product development and assessment of *in vivo* performance. Dr. Longest and I have collaborated on using this quantitative analytical design (QAD) to guide the development of pharmaceutical inhalers and this successful approach will be implemented for the device design component of the proposed project.

This current grant application will build on this collaborative relationship by taking forward the development of the excipient enhanced growth concept (EEG) for a novel pediatric aerosol delivery application to deliver surfactants to the lungs of neonates. The specific practical experience that I have relating to dry powder formulation, inhaler design, in vitro test method development, pharmaceutical analytical method development, and aerosol characterization will be essential for the success of this project. I have additional experience of protein formulation (US patent granted describing a novel insulin aerosol formulation) and protein spray drying. Also of relevance, for the past 5 years I have taught surface science and surfactant chemistry in our Professional curriculum at VCU. The submicrometer combination particle production methods used in the EEG approach were developed during our recent R21 grant as proof of concept studies, which resulted in six publications and a patent application. Using the EEG approach was shown to nearly eliminate mouth-throat depositional loss and produce aerosol size increase sufficient for full lung retention using both spray and dry

powder devices. We have also demonstrated our ability to improve delivery of aerosols during NIV to adult patients during our R01 project, which resulted in 21 publications and a number of patent applications. Importantly, this work was recognized as improving aerosol delivery during NIV in a recent editorial (Resp Care, 2014; 59; 1608-1610). Our laboratories are well equipped to carry out the necessary *in vitro* and CFD studies described in this project.

1. Forbes RT, Davis KG, Hindle M, Clarke JG, Maas J. Water vapor sorption studies on the physical stability of a series of spray-dried protein/sugar powders for inhalation. *J Pharm Sci.* 1998 Nov; 87(11):1316-21. PubMed PMID: 9811483.
2. Hindle M and Cox K.A., Liquid aerosol formulations containing insulin and aerosol generating devices and methods for generating insulin aerosols. US Patent # 7,683,029. Issued 2010.
3. Longest PW, Azimi M, Golshahi L, Hindle, M. Improving aerosol drug delivery during invasive mechanical ventilation with redesigned components. *Respiratory Care.* 2014; 59(5): 686-698. PMID: 21410327; PMCID: PMC3123840
4. Longest PW, Golshahi L, Behara SRB, Tian G, Farkas DR, Hindle M. Efficient nose-to-lung (N2L) aerosol delivery with a dry powder inhaler. *J Aerosol Med Pulm Drug Deliv.* 2015 Jun;28(3):189-201. doi: 10.1089/jamp.2014.1158. Epub 2014 Sep 5. PMID: 25192072 PMCID: PMC4559155.

B. Positions and Honors

Positions and Employment

1995 - 1996	Lecturer in Pharmaceutics, University of Bradford, Bradford
1997 - 2016	Associate Professor, Virginia Commonwealth University, Richmond, VA
2016 - present	Peter R. Byron Distinguished Professor, Virginia Commonwealth University, VA
2003 - 2005	Graduate Program Director, Virginia Commonwealth University, Dept of Pharmaceutics, Richmond , VA

Other Experience and Professional Memberships

2013 -	Member, The Aerosol Society
1989 -	Registered Pharmacist in the United Kingdom
1989 - 2010	Member, Royal Pharmaceutical Society of Great Britain
1991 -	Member, American Association of Pharmaceutical Scientists

Honors

2009	Scientific Consultant, Meritage Pharma, Inc
2014-2016	Co-chair, NIH SEP, Small Business Respiratory Sciences
2015	Chair, NIH NAID Special Emphasis Panel
2016	Awarded endowed Peter R. Byron Distinguished Professorship

C. Contributions to Science

1. *In vitro and in vivo regulatory method development*

I have contributed to the development of regulatory and compendial methods of assessing pharmaceutical inhalers. During my doctoral studies, I developed a novel, non-invasive method of estimating *in vivo* pulmonary bioavailability of inhalation aerosols which has been utilized by a number of pharmaceutical companies in support of produce licensing in Europe. This simple approach of taking a urine sample 30 minutes following inhalation allowed estimates of the relative pulmonary bioavailability of the aerosol without interference from the oral fraction of the inhaled dose and therefore only reflected the dose delivered to the airways. Studies were performed to investigate the effects of inhalation technique, spacer devices and generic devices on the relative bioavailability of inhaled albuterol. In collaboration with Dr. Peter Byron, I also developed a realistic method of measuring the *in vitro* delivered dose from dry powder inhalers. This method was subsequently adopted by the USP as the official method for dry powder inhaler testing.

1. Hindle M, Chrystyn H. Determination of the relative bioavailability of salbutamol to the lung following inhalation. Br J Clin Pharmacol. 1992; 34(4): 311-5. PMID: 1457264; PMCID: PMC1381412.
2. Hindle M, Newton DA, Chrystyn H. Investigations of an optimal inhaler technique with the use of urinary salbutamol excretion as a measure of relative bioavailability to the lung. Thorax. 1993; 48(6): 607-10. PMID: 8346489; PMCID: PMC464579.
3. Hindle M, Peers EM, Parry-Billings M, Chrystyn H. Relative bioavailability of salbutamol to the lung following inhalation via a novel dry powder inhaler and a standard metered dose inhaler. Br J Clin Pharmacol. 1997; 43(3): 336-8. PMID: 9088593; PMCID: PMC2042743.
4. Hindle M, Byron PR. Dose emissions from dry powder inhalers. Int J Pharm. 1995; 116(2): 169-177.

2. Investigation of *in vitro* and *in vivo* correlations for inhaled aerosols

The development of realistic in vitro methods to investigate the performance of inhaled aerosol products is of interest to both the regulators and the pharmaceutical industry. From the industry perspective, methods of establishing an *in vitro – in vivo* correlation are attractive to prevent unnecessary clinical studies. Similarly, the regulatory agencies are also interested in methods that accurately characterize inhaler performance in an *in vitro* environment. Our group has pioneered the use *in vitro* aerosol drug deposition testing using realistic airway geometries and flow profiles to establish *in vitro – in vivo* correlations for inhaled drug products in work sponsored by the US FDA. These investigations have also included *in silico* computational fluid dynamic simulations which have been developed by Dr. Longest and his group. Our combined efforts in this area have produced comprehensive studies examining the *in vitro* performance of a series of inhaled products and produced correlations *in silico* using a stochastic individual pathway modeling approach to simulate whole lung aerosol deposition that are in close agreement with in vivo regional lung deposition data.

1. Tian G, Hindle M, Lee S, Longest PW. Validating CFD predictions of pharmaceutical aerosol deposition with in vivo data. *Pharmaceutical Research.* 2015; 32(10): 3170-3187. PMID: 25944585
2. Longest PW, Tian G, Walenga RL, Hindle M. Comparing MDI and DPI aerosol deposition using in vitro experiments and a new stochastic individual path (SIP) model of the conducting airways. Pharm Res. 2012; 29(6):1670-88. doi: 10.1007/s11095-012-0691-y. PMID: 22290350.
3. Longest PW, Tian G, Khajeh-Hosseini-Dalasm N, Hindle M. Validating whole-airway CFD predictions of DPI aerosol deposition at multiple flow rates. J Aerosol Med Pulm Drug Deliv. 2016; 29(3):461-481 doi: 10.1089/jamp.2015.1281. PMID: 27082824.
4. Delvadia R, Hindle M, Longest PW, Byron PR. In vitro tests for aerosol deposition II: IVIVCs for different dry powder inhalers in normal adults. J Aerosol Med Pulm Drug Deliv. 2013; 26(3):138-44. doi: 10.1089/jamp.2012.0975. Epub 2012 Sep 4. PMID: 22947131.

3. Improvements in aerosol drug delivery during noninvasive ventilation

Drug delivery during noninvasive ventilation is characterized by high deposition losses in the ventilatory tubing and low drug delivery to the lungs. Our group, in collaboration with Dr. Longest, has developed methods of improving aerosol delivery during high flow nasal cannula therapy, low flow oxygen therapy and noninvasive positive pressure ventilation. Our approaches have been tested both *in silico* (Dr Longest) and *in vitro* by my group. Our methods have been tested using realistic testing conditions including airway geometry models and breathing profiles and have been compared to the current standard of care. The new approach of controlled condensational growth has been successful at improving lung delivery rates for orally-administered aerosols and for nose-to-lung delivery of both powder aerosols and nebulizer generated aerosols during NIV. For example, in a system employing a novel aerosol mixer-heater system combined with a mesh nebulizer, synchronizing the aerosol delivery with patient breathing was important to achieve high efficiency aerosol delivery (>70%) in an adult high flow nasal cannula (HFNC) system compared to the current standard of care which delivers less than 5% of the aerosol to the patient.

1. Walenga RL, Tian G, Hindle M, Yelverton J, Dodson K, Longest PW. Variability in Nose-to-Lung Aerosol Delivery.J Aerosol Sci. 2014; 78: 11-29. PMID: 25308992; PMCID: PMC4187112.

2. Tian G, Hindle M, Longest PW. Targeted Lung Delivery of Nasally Administered Aerosols. Aerosol Sci Technol. 2014; 48(4): 434-449. PMID: 24932058; PMCID: PMC4051279.
3. Golshahi L, Tian G, Azimi M, Son YJ, Walenga R, Longest PW, Hindle M. The use of condensational growth methods for efficient drug delivery to the lungs during noninvasive ventilation high flow therapy. Pharm Res. 2013; 30(11): 2917-30. doi: 10.1007/s11095-013-1123-3. Epub 2013 Jun 26. PMID: 23801087; PMCID: PMC3800269.
4. Longest PW, Golshahi L, Hindle M. Improving pharmaceutical aerosol delivery during noninvasive ventilation: effects of streamlined components. Ann Biomed Eng. 2013; 41(6): 1217-32. doi: 10.1007/s10439-013-0759-9. Epub 2013 Feb 20. PMID: 23423706; PMCID: PMC3647043.

4. Development of the next generation dry powder formulations and devices for inhalation.

Traditionally inhaled powder formulations employ cohesive micronized drug blended with a lactose carrier which is dispersed by a dry powder inhaler resulting in high oropharyngeal deposition losses of the aerosol and low delivery efficiency to the site of action in the lungs. The approach that we have pioneered at VCU to improve the performance of powder inhaler devices is to develop novel highly dispersible submicrometer combination particle powder formulations using a spray drying technique. This approach has been successfully employed to generate stable powder formulations for a number of drugs including albuterol sulfate, terbutaline sulfate, azithromycin, tobramycin, and commercial Survanta lung surfactant. The submicrometer particles are easily dispersible due to the presence of leucine, an amino acid, which acts as a dispersion enhancer. The submicrometer size enables the particles to avoid depositional losses in the mouth-throat region during inhalation and ensures efficient delivery to the lungs. The inclusion of a hygroscopic excipient in the combination particle enables particle growth in the humid airways of the lung increasing the size of the submicrometer sized particle to cause its deposition and prevent exhalation. In combination with the powder formulations, our group in collaboration with Dr. Worth Longest, has developed a series of dry powder inhalers that have been optimized to generate aerosols that offer significant improvements in aerosol performance compared to current commercial devices. These studies have generated numerous publications and a patent application is pending describing the devices and formulations.

1. Son YJ, Longest PW, Tian G, Hindle M. Evaluation and modification of commercial dry powder inhalers for the aerosolization of a submicrometer excipient enhanced growth (EEG) formulation. *Eur J Pharm Sci*. 2013; 49: 390-399. PMID: 23608613; PMCID: PMC3744372.
2. Son YJ, Longest PW, Hindle M. Aerosolization characteristics of dry powder inhaler formulations for the excipient enhanced growth (EEG) application: Effect of spray drying process conditions on aerosol performance. *Int J Pharm.* 2013; 443: 137-145. PMID: 23313343; PMCID: PMC3584634.
3. Behara SRB, Longest PW, Farkas DR, Hindle M. Development and comparison of new high-efficiency dry powder inhalers for carrier-free formulations. J Pharm Sci. 2014; 103(2): 465-77. doi: 10.1002/jps.23775. Epub 2013 Dec 4. PMID: 24307605; PMCID: PMC3947484.
4. Behara SRB, Farkas DR, Hindle M, Longest PW. Development of a high efficiency dry powder inhaler: effects of capsule chamber design and inhaler surface modifications. Pharm Res. 2014; 31(2): 360-72. doi: 10.1007/s11095-013-1165-6. Epub 2013 Aug 16. PMID: 23949304; PMCID: PMC3946921.

A full bibliography can be obtained at

http://www.ncbi.nlm.nih.gov/sites/myncbi/michael.hindle.1/bibliography/40663661/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

Hindle (PI) 09/10/14 – 08/31/2017
US FDA U01
Title: Development of Clinically Relevant in Vitro Performance Test for Generic OIDPs

Objective: A comparison of the use of realistic mouth geometries and realistic inhalation flow rates to characterize pharmaceutical aerosols.

Longest (PI), Hindle (PI), Mossi (I), and Dodson (I) 6/1/11 – 3/31/17
NIH/NHLBI R01
Title: Effective Delivery of Pharmaceutical Aerosols during Non-Invasive Ventilation
Objective: The objective of this study is to develop pharmaceutical delivery systems for adults that can significantly enhance aerosol deposition in the lungs during non-invasive ventilation (NIV) using a condensational growth approach.

Hindle (Co-PI), Longest (Co-PI) 9/30/13- 9/29/17
University of Florida (Subcontract from US FDA contract # HHSF223201310223C)
Title: Applicability of in silico, in vitro and pharmacokinetics studies to elucidate critical API changes in nasal suspension formulations.
Objective: The objective of this study is to develop in vitro and CFD methods to characterize nasal spray suspension products.

Completed Research Support

Longest (PI) and Hindle (PI) 7/1/10 – 6/30/13
NIH/NHLBI R21
Title: Excipient Enhanced Aerosol Particle Formulations and Inhaler Development for Improved Pulmonary Drug Delivery
Objective: The overall goal of this project was to develop a novel technology for the efficient delivery of inhaled nanoparticles to adults that minimizes deposition in the mouth and throat while maximizing delivery to the lungs.

Longest (PI), Hindle (I), Byron (I) 9/15/12 – 9/14/16
US FDA U01
Title: Predictive Lung Deposition Models for Safety and Efficacy of Orally Inhaled Drugs
Objective: The objective of this study is to develop combination CFD and *in vitro* adult airway models that can predict the *in vivo* deposition of orally inhaled drugs across a population.

Longest (PI), Hindle (I) and Tepper 8/5/12 – 4/30/16
NIH/NHLBI R21
Title: Nanoaerosols from Wick Electrospray for Improved Drug Delivery to Infants
Objective: This project seeks to use charged nanoparticles to enhance respiratory drug delivery to mechanically ventilated infants.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Bruce K Rubin	Jessie Ball duPont Distinguished Professor and Chairman, Dept of Pediatrics Professor of Biomedical Engineering
eRA COMMONS USER NAME (credential, e.g., agency login) eRACommons	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Tulane University, New Orleans USA	B. S.	06/1975	Physics, Mathematics
Tulane University, New Orleans USA	M. Engr.	06/1977	Biomedical Engineering
Tulane University, New Orleans USA	MD	06/1979	Medicine
Oxford University, UK	Post Doc	06/1980	Paediatric bioengineering
The Hospital for Sick Children, Toronto, Canada	Residency	06/1981	Paediatrics
The Hospital for Sick Children, Toronto, Canada	Fellowship	06/1983	Paediatric respirology
Wake Forest University, Winston-Salem, NC	MBA	2004	Management

A. Personal Statement and Contributions to Science

As a pediatric pulmonologist and an engineer I have spent the past 30 years studying airway biomaterials, developing and testing medical aerosols, and creating animal, and cell and tissue culture models of airway disease. Our discoveries have led to 9 patents, I have trained more than 50 graduate students, fellows, and post-doctoral candidates in my laboratory and conducted dozens of investigator-initiated clinical trials based on discoveries in my laboratory. My lab has the capacity to grow and differentiate airways, study cell-cell and cell signaling interactions, test hypotheses in unique animal models, and to conduct randomized controlled trials.

B. Positions and Honors

Positions and Employment

1980–1981 Tulane University, New Orleans — Pediatric resident
1981-1982 The Hospital for Sick Children, Toronto Ontario — Post core resident
1982-1983 The Hospital for Sick Children, Toronto Ontario — Fellow in Paediatric Respirology
1983-1987 Assistant Professor, Queen's University at Kingston, Ontario Department of Paediatrics
1987-1991 Assistant Professor, Alberta Heritage Foundation and University of Alberta Dept. of Pediatrics
1991-1997 St. Louis University School of Medicine, Professor of Pediatrics with tenure
1997-2009 Wake Forest University School of Medicine, Professor & Vice Chair for Research Dept. of Pediatrics
2009- present Virginia Commonwealth University, Jessie Ball duPont Distinguished Professor and Chair of the Dept of Pediatrics, Professor of Biomedical Engineering, Adjunct Professor of Physiology and Biophysics, Microbiology and Immunology

Honors, Awards, Professional memberships

Rhodes Scholar 1978-80
American College of Chest Physicians Young Investigator Award 1989
Alfred Soffer Research Award 1990
Du Pont Critical Care Research Award 1990
Alfred Soffer Award for Editorial Excellence 2004
CIPP Prix Extraordinaire; 2008
AARC Forrest Bird Award for Lifetime Scientific Achievement 2008
36th Annual Donald Eagan memorial speaker 2009
27th Phil Kittredge Memorial lecturer 2011
Jimmy A Young Medal 2012
Fellow of the American Academy of Pediatrics
American Association for Respiratory Care
Royal College of Physicians and Surgeons of Canada

Elected member of American Pediatric Society, Society for Pediatric Research
Currently Associate Editor of *Chest*, *Clinical Pulmonary Medicine*, *Respiratory Care*, and *Paediatric Respiratory Reviews.* Also editorial Board member of *Journal of Aerosol Medicine, European Respiratory Journal, Pediatric Pulmonology, Journal of the COPD Foundation, Current Respiratory Medicine Reviews, American Journal of Respiratory and Critical Care Medicine, Expert Reviews in Pulmonary Medicine, Pediatric Pulmonology Chair F1000 Medicine*

C. Contribution to Science

URL for partial list of publication entered into PubMed. NB: some publications listing me a B Rubin (rather than BK Rubin) are not listed here.
As of January 2017: 273 original research papers, H (Hirsch) index 51; i10 Index 174
http://www.ncbi.nlm.nih.gov/pubmed/?term=rubin+bk
A more complete list (including publications without the middle initial) is available at Google Scholar:
https://scholar.google.com/citations?user=U4slVWgAAAAJ&hl=en

[1] *Determining the biophysical properties of cystic fibrosis and other respiratory secretions*
As a materials engineer in Alberta, Canada, I studied how oil flows in pipelines under adverse conditions. As a pulmonary physician I had the epiphany that if oil was mucus and pipelines were airways, some of these techniques and models could be informative. My group developed novel methods for measuring mucus rheology, adhesiveness, and transport and, over the past 30 years, we have refined these techniques to dramatically enhance our understanding of mucus transport in heath and disease. Selected references (from over 70 on this topic):

1. Voynow JA, **Rubin BK**. Mucus, mucins, and sputum. Chest 2009;135:505-512.
2. **Rubin BK**, Ramirez O, King M. Mucus-depleted frog palate as a model for the study of mucociliary clearance. J Appl Physiol 1990; 69:424-429
3. Albers GM, Tomkiewicz RP, May MK, Ramirez OE, **Rubin BK**. Ring distraction technique for measuring the surface tension of sputum and relationship of the work of adhesion to clearability. J Appl Physiol 1996;81:2690-95
4. Bush A, Payne D, Pike S, Jenkins G, Henke MO, **Rubin BK**. Mucus properties in children with primary ciliary dyskinesia: Comparison with cystic fibrosis. Chest 2006;129:118-123
5. Schmidt JH, Priftis K, Henke MO, **Rubin BK**. Secretory hyperresponsiveness and mucus hypersecretion. CHEST 2014;146:496-507

[2] *We have also used these techniques to study physiotherapy and mechanical means of sputum clearance as well as to develop new drug therapies*. This led to our discovery of airway surfactant as an essential mucokinetic and to clinical applications of surfactant including a patent issued. Selected publications:

1. **Rubin BK**, Ramirez O, King M. Mucus rheology and transport in neonatal respiratory distress syndrome and the effect of surfactant therapy. Chest. 1992;101(4):10808-5..
2. De Sanctis GT, Tomkiewicz RP, **Rubin BK**, Schürch S, King M. Exogenous surfactant enhances mucociliary clearance in the anaesthetized dog. Eur Respir J. 1994;7:1616-21.
3. Anzueto A, Jubran A, Ohar JA, Piquette CA, Rennard SI, Colice G, Pattishall EN, Barrett J, Engle M, Perret KA, **Rubin BK**. Effects of aerosolized surfactant in patients with stable chronic bronchitis: a prospective randomized controlled trial.J Am Med Assoc 1997;278(17):1426-31.
4. Kempainen RR, Williams CB, Hazelwood A, **Rubin BK**, Milla CE. Comparison of high-frequency chest wall oscillation with differing waveforms for airway clearance in cystic fibrosis. Chest 2007;132:1227-32
5. Kater A, Henke MO, Rubin **BK.** The role of DNA and actin polymers on the polymer structure and rheology of cystic fibrosis sputum and depolymerization by gelsolin or thymosin Beta 4. Ann N Y Acad Sci. 2007;1112:140-53

[3] *Developing novel techniques for aerosol delivery to children.* The study of mucus flow in airways also lends itself to aerosol flow. We have studied aerosol therapy (including mannitol) in health and disease for over 30 years with a particular emphasis on clinical applications of aerosol therapy in children. Selected publications (from more than 30 in this field)

1. **Rubin BK**, Fink JB. The delivery of inhaled medication to the young child. Pediatr Clinics N. America 2003;50:717-31.
2. **Rubin BK**, Williams RW. Aerosolized antibiotics for non-CF bronchiectasis. Respiration 2014;22:177-184
3. Daviskas, E Anderson SD, Gomes K, Briffa P, Cochrane B, Chan H-Kim, Young IH, **Rubin BK.** Inhaled mannitol for the treatment of mucociliary dysfunction in patients with bronchiectasis - Effect on lung function, health status and sputum. Respirology 2005;10:46-56.
4. **Rubin BK.** Aerosol medications for treatment of mucus clearance disorders. Respir Care. 2015;60(6):825-32.
5. **Rubin BK**, Williams RW. Emerging aerosol drug delivery strategies: From bench to clinic. Adv Drug Deliv Rev. 2014;75:141-48.

[4] *Macrolides as immunomodulatory medications.* In 1993, I learned from Dr. Kishioka (a post doc in my lab), about the effectiveness of macrolide antibiotics in treating diffuse panbronchiolitis, a CF like disease seem almost exclusively in East Asia. That year, I was the first in North America to use oral macrolides at low dose to treat a desperately ill young woman with CF. Her dramatic response led to 20 years of studying the effectiveness of macrolide therapy in chronic lung disease, the mechanism of action of these medication especially on neutrophil dominant inflammation, and the development of novel therapies based on these mechanisms of action. Selected publication (from over 40)

1. **Rubin BK**, Druce H, Ramirez OE, Palmer R. The effect of clarithromycin on mucus properties in healthy subjects and in patients with acute purulent rhinitis. Am J Respir Crit Care Med 1997;115:2018-23
2. Shinkai M, Foster G, **Rubin BK**. Macrolide antibiotics modulate ERK phosphorylation and IL-8 and GM-CSF production by human bronchial epithelial cells. Am J Physiol Lung Cell Molec Physiol 2006;290:L75-85.
3. Tanabe T, Kanoh S, Tsushima K, Yamazaki Y, Kubo K, **Rubin BK**. Clarithromycin inhibits interleukin-13-induced goblet cell hyperplasia in human airway cells. Am J Respir Cell Mol Biol. 2011,45;1075-83
4. Shinkai M, Henke MO, **Rubin BK**. Macrolide antibiotics as immunomodulatory medications: Proposed mechanisms of action Pharmacology and Therapeutics 2008;117:393-405.
5. Kanoh S, Tanabe T, **Rubin BK**. Dapsone inhibits IL-8 secretion from human bronchial epithelial cells stimulated with LPS and resolves airway inflammation in the ferret. CHEST 2011;140:980-90.

[5] *Secretory hyperresponsiveness as a distinct response to airway inflammation.* Although it has been established that mucus secretion is component of CF, asthma, COPD, and other inflammatory airway diseases, the cell and molecular mechanisms for hyperseretion are still being defined. We have contributed to this field with over 20 publications defining "secretory hyperresponsiveness" as the clinical phenotype of hypersecretion-dominant airway disease. Selected publications:

1. Okamoto K, Kim JS. **Rubin BK**. Secretory phospholipases A2 stimulate mucus secretion, induce airway inflammation, and produce secretory hyperresponsiveness to neutrophil elastase in the ferret trachea. Am J Physiol Lung Cell and Molec Physiol. 2007;292:L62-67.
2. Kanoh S, Tanabe T, **Rubin BK**. IL-13-induced MUC5AC production and goblet cell differentiation is steroid resistant in human airway cells. Clin Exp Allergy 2011;41:1747-56.

3. Tanabe T, Shimokawaji T, Kanoh S, **Rubin BK.** Secretory phospholipases A_2 are secreted from ciliated cells and increase mucin and eicosanoid secretion from goblet cells. CHEST 2015;147:1599-1609
4. Tokita E, Tanabe T, Asano K, Suzaki H, **Rubin BK**. Club cell 10-kDa protein attenuates airway mucus hypersecretion and inflammation. Eur Respir J. 2014;44(4):1002-10
5. Tanabe T, Shimokawaji T, Kanoh S, **Rubin BK**. IL-33 stimulates CXCL8/IL-8 secretion in goblet cells but not normally differentiated airway cells. Clin Exp Allergy. 2014;44:540-52.

D. Current Research Support

Private Source

Aztreonam aerosol to treat cystic fibrosis nasal disease
Role: PI
Objective is to use a novel therapy, nasal antibiotics, and a novel nasal delivery device to treat chronic sinonasal infection in CF with an aim to reducing hospitalizations.

Private Source

Title: Finding new therapies for cystic fibrosis
Role: PI
Objective is to support a graduate student evaluating role of Tissue Factor in CF mucus hypersecretion.

Private Source

The effect of nocturnal high flow humidity on quality of life, pulmonary function and mucus properties in hospitalized patients with cystic fibrosis
Role: PI
Objective is to evaluate a novel therapy, high flow and high humidity, on mucus clearance and sleep quality in hospitalized subjects with acuter exacerbations of CF lung disease.

Private Source

The effect of dapsone aerosol in the inflamed ferret airway using a novel formulation and delivery device
Role: PI
Objective is to evaluate a new drug, developed in the PI's lab, to treat inflammatory secretory hyperresponsiveness.

Private Source

The anti-inflammatory effects of tiotropium bromide in IL-13 transformed human airway
Role: PI
Objective is to evaluate tiotropium, an anticholinergic, on TH2 dominant inflammation and goblet cell transformation in cultured human airways.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

NAME: Rebecca L. Heise

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Assistant Professor of Biomedical Engineering

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Carnegie Mellon University, Pittsburgh, PA	B.S.	05/2003	Biomedical Engineering
Carnegie Mellon University, Pittsburgh, PA	B.S.	05/2003	Chemical Engineering
University of Pittsburgh, Pittsburgh, PA	Ph.D.	08/2008	Bioengineering
National Institute of Environmental Health Sciences	Postdoctoral	10/2010	Respiratory Biology

A. Personal Statement

My research career is devoted to understanding the mechanobiology of cells and their extracellular matrix (ECM) in pathologies of dynamic organ systems, and to use this information to repair the lung or prevent lung disease. My research laboratory has two current focuses 1.To investigate the mechanisms by which cells of the lung sense and respond to mechanical forces in health and disease and 2. To develop novel regenerative medicine strategies for lung repair. I have expertise in mechanisms of cellular response to injury and analysis of signaling pathways in lung epithelia and my previous research experience and training was in biomechanics and regenerative medicine with a focus on progenitor cell response to naturally derived biomaterials. All of my background and previous research have fully prepared me for my ongoing projects. Personal Info However, upon returning to the field, I immediately resumed my research projects and collaborations and successfully competed for extramural support. Though this caused a gap in my publication record, I have maintained participation in conference meetings and have 3 manuscripts accepted within the last year, with four additional manuscripts under review. My laboratory has been working with rodent lung injury models for the past four years. Furthermore, I have been working in the arena of drug delivery and have published on delivering hydrogels to rat lungs. In the proposed work, I will be assisting the PIs in Specific Aim 1 to assess efficacy and dosing of the EEG surfactant formulation in an animal model. We will utilize a rat surfactant depletion model for this work. We will deliver the surfactant therapies with a microsprayer and assess pulmonary mechanics, blood oxygenation, and inflammatory markers in the rats. I have been working with the PIs for the past year in developing this project. PI Longest and I have a collaborative internal award together to provide preliminary data for this proposal. I will make all the resources in my laboratory available to the PIs as we complete this study.

1. Herbert J, Valentine M, Patel P, Pidaparti R, Reynolds A, **Heise RL.** "The Role of ER Stress in Aging In vitro and In vivo Models of Ventilator Induced Lung Injury." American Journal of Respiratory and Critical Care Medicine 2016, Volume 193
2. Herbert JA, Valentine MS, Saravanan N, Schneck MB, Pidaparti R, Fowler AA 3rd, Reynolds AM, **Heise RL**. "Conservative Fluid Management Prevents Age-Associated Ventilator Induced Mortality." Exp Gerontol. 2016 May 14. pii: S0531-5565(16)30140-1. doi: 10.1016/j.exger.2016.05.005. PubMed PMID: 27188767.
3. Pouliot RA, Link PA, Mikhaiel NS, Schneck MB, Valentine MS, Kamga Gninzeko FJ, Herbert JA, Sakagami M, **Heise RL**. "Development and characterization of a naturally derived lung extracellular matrix hydrogel." J Biomed Mater Res A. 2016 Mar 25. doi: 10.1002/jbm.a.35726. PMID: 27012815.

B. Positions and Honors.

Positions

2010-Present **Assistant Professor**, Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA

2014-Present **Assistant Professor**, Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA

Other Experience and Professional Memberships

1999-- Biomedical Engineering Society

2008-- American Thoracic Society

Honors

2005 ORAU Delegate, 55th Annual Lindau Meeting of Nobel Laureates and Students

2007 McGowan Trainee Career Advancement Program Travel Award

2007 Finalist, ASME Summer Bioengineering Conference PhD Competition

2007 Graduate Research Award, Biomedical Engineering Society

2007 Second Annual NIH National Graduate Student Research Festival

2011 Qimonda Discretionary Funds Award

2012 VCU Pew Scholars in the Biomedical Sciences Nominee

2013 VCU Pew Scholars in the Biomedical Sciences Nominee

2013 Blavatenik Young Scientists Nominee

2014 NSF CAREER Award

C. Contributions to Science

1. I have been working on cellular response to naturally--derived ECM scaffolds since I began graduate school in 2003. My dissertation work in tissue engineering the urinary bladder is a key area of significant contribution. I showed, for the first time, that mechanical stimulation and growth factors could be harnessed to promote smooth muscle cell elastin production. The elastin produced yielded a more functional replacement for urologic tissues because it provided superior mechanical properties compared with the extracellular matrix scaffold alone. This work has broader applications for regenerative strategies of vasculature and the lung. The recent paper from my independent lab shows how we developed and characterized a lung ECM hydrogel for cellular delivery to the emphysematous lung.

1a. Link, P. A., Pouliot, R. A., Mikhaiel, N. S., Young, B. M., **Heise, R. L.** Tunable Hydrogels from Pulmonary Extracellular Matrix for 3D Cell Culture. *J. Vis. Exp.* (119), e55094, doi:10.3791/55094 (2017). PMID not yet available

1b. Pouliot RA, Link PA, Mikhaiel NS, Schneck MB, Valentine MS, Kamga Gninzeko FJ, Herbert JA, Sakagami M, **Heise RL**. "Development and characterization of a naturally derived lung extracellular matrix hydrogel." J Biomed Mater Res A. 2016 Mar 25. doi: 10.1002/jbm.a.35726. PMID: 27012815.

1c. **Heise RL,** Ivanova J, Parekh A, and Sacks MS. "Generating Elastin-Rich SIS-Based Smooth Muscle Constructs Utilizing Exogenous Growth Factors and Cyclic Mechanical Stimulation." (2009) Tissue Engineering. Dec;15(12):3951-60. PMID: 19569874

1d. Long RA (Maiden Name), Nagatomi. J, Chancellor MB, and Sacks MS. "The Role of MMP-I Up-regulation in the Increased Compliance in Muscle-derived Stem Cell-seeded Small Intestinal Submucosa." (2006) Biomaterials. Apr;27(11):2398-404. PMID: 16337680

2. Another important contribution to science has been my work on lung epithelial injury. I found that mechanical strain caused epithelial to mesenchymal transition in alveolar type II epithelium through

hyaluronan activation of innate immunity. I furthered these concepts in examining the role of hyaluronan in bronchial epithelial cells.

2a. Stober VP, Szczesniak C, Childress Q, **Heise RL**, Bortner C, Hollingsworth JW, Neuringer IP, Palmer SM, Garantziotis S. "Bronchial epithelial injury in the context of alloimmunity promotes lymphocytic bronchiolitis through hyaluronan expression." Am J Physiol Lung Cell Mol Physiol. 2014 Jun 1;306(11):L1045-55. doi: 10.1152/ajplung.00353.2013. Epub 2014 Apr 18. PubMed PMID: 24748604; PubMed Central PMCID: PMC4042191.

2b. Heise RL, Stober V, Cheluvaraju C, Hollingsworth JW, and Garantziotis S. "Mechanical Stretch Induces Epithelial to Mesenchymal Transition of Alveolar Type II Epithelium Through Hyaluronan Expression and Innate Immune Activation." (2011) Journal of Biological Chemistry. May 20;286(20):17435-44. PMID: 21398522

3. My current work in ventilator induced lung injury is poised to make a large impact on the field of mechanical stretch induced lung injury. Our collaborative efforts are combining experiments and modeling to assess the role of the waveform and strain levels in ventilator induced lung injury in the aging population. We have published two papers on these efforts with one additional paper under review. We have discovered that the aging lung is prone to pulmonary edema, and that ventilator associated mortality may be avoided in aged mice with conservative fluid management.

3a. Herbert JA, Valentine MS, Saravanan N, Schneck MB, Pidaparti R, Fowler AA 3rd, Reynolds AM, **Heise RL**. "Conservative Fluid Management Prevents Age-Associated Ventilator Induced Mortality." Exp Gerontol. 2016 May 14. pii: S0531-5565(16)30140-1. doi: 10.1016/j.exger.2016.05.005. PubMed PMID: 27188767.

3b. R. M. Pidaparti, M. Burnette, **Heise RL** and Reynolds A. "Analysis of Stress Environment in the Alveolar Sac Model, Journal of Biomedical Science and Engineering, DOI: 10.4236/jbise.2013.69110, pp. 901-907, September 20, 2013.

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47074965/?sort=date&direction=ascending

D. Research Support.

Current Support

7/2016-12/2017 VCU PERQ "Developing a Practical Delivery Device and Animal Proof-of-Concept Data for a New Inhaled Surfactant Therapy Developed at VCU"
Role: co-PI with P. Worth Longest. Major Goal: To develop EEG formulation delivery device and proof of concept data.

02/2014-01/2019 NSF CMMI-1351162 "CAREER: Propagation of Lung Fibrosis through Mechanotransduction"
Role: PI Major goal: The research objective for this proposal is to test the hypothesis that mechanical stretch of epithelial cells propagates tissue fibrosis through epithelial to mesenchymal transition.

08/2012-05/2017 NIH 1R01AG041823-01A1 (MPI) "Age Dependent Mechanical Ventilator-Induced Inflammation: Modeling & Experiments" Role: PI. Major goal: To develop a multi-scale hybrid model of age-related inflammation in the lung under mechanical ventilation.

Completed Support

07/2013-11/2015 Commonwealth Health Research Board "Development of Extracellular Matrix Hydrogels for Lung Regeneration" Role: PI Sakagami (Co-I)
Major goal: To develop and characterize ECM hydrogels for cell delivery in a model of chronic obstructive pulmonary disease.

06/2011-06/2015 Private Source "Primary cilia mechanotransduction in non-small cell lung cancer." Role: PI

Major goal: To investigate primary cilia and the sonic hedgehog pathway in tumor progression in non-small cell lung adenocarcinoma.

2011-2013 Virginia Commonwealth University Presidential Research Incentive Program Award. "Mechanisms of mechanical stretch induction of alveolar epithelial to mesenchymal transition." Role: PI.

2011-2012 [Private Source] "Primary Cilia Mechanical Signaling in Non-Small Cell Lung Cancer" Role: PI

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Willson, Douglas F.

eRA COMMONS USER NAME (credential, e.g., agency login): [eRA Commons User Name]

POSITION TITLE: Professor, Pediatrics and Anesthesia

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Kenyon College, Gambier, Ohio		1971	Biology
Cornell University, Ithaca, New York	B.A.	1973	Biology
Downstate Medical Center, State University of New York, Brooklyn, New York	M.D.	1977	Medicine
North Carolina Memorial Hospital (Chapel Hill, N.C.):		1980	Pediatric Residency
North Carolina Memorial Hospital		1981	Chief Resident, Pediatrics
North Carolina Memorial Hospital		1979 1983	Anesthesia Residency
The Hospital for Sick Children, Toronto, Canada		1983	Pediatric ICU fellowship

A. Personal Statement

My training as a pediatric intensivist and anesthesiologist has led to my focus on acute lung injury and respiratory support throughout my career. My primary research interest has been in the use of surfactant as therapy in pediatric ARDS and I have successfully conducted several multi-institutional and one international study on this topic. I believe that I am an ideal clinical collaborator for this project because of my extensive clinical experience in lung injury in children, my experience as a principle investigator for several large multi-center clinical trials, and my expertise in surfactant, as highlighted in the following publications.

1. **Willson DF**, Truwit J, Conaway M, Traul C, Egan EE. The Adult Calfactant in Acute Respiratory Distress Syndrome (CARDS) Trial. Chest 2015; 148:356-64. PMID 25855884
2. **Willson DF**, Thomas NJ, Tamburro R, Truemper E, Truwit J, Conaway M, Traul C, Egan E. The Pediatric Calfactant in Acute Respiratory Distress Syndrome (CARDS) Trial. Pediatr Crit Care Med 2013: 14:657-65. PMID 23846250
3. **Willson DF**, Thomas NJ, Tamburro R, Truemper E, Truwit J, Conaway M, Traul C, Egan E. The Relationship of Fluid Administration to Outcome in the Pediatric Calfactant in Acute Respiratory Distress Syndrome (CARDS) Trial. Pediatr Crit Care Med 2013:14:666-72. PMID 23925143

B. Positions and Honors.

1983-1989 Assistant Professor of Anesthesiology and Pediatrics, University of Virginia Health Sciences Center, Charlottesville, VA
1989-1991 Assistant Professor of Anesthesiology and Pediatrics, Children's Hospital of Cincinnati, University of Cincinnati; Cincinnati, OH
1991-2007 Associate Professor of Pediatrics and Anesthesiology, University of Virginia Health Sciences Center; Charlottesville, VA (Tenured 1998).
1991- 2008 Director, Pediatric ICU, and Division of Pediatric Critical Care, University of Virginia Health Sciences Center; Charlottesville, VA
2004-2009 Chairman, Collaborative Pediatric Critical Care Research Network (of the NICHD)
2007-2013 Professor of Pediatrics and Anesthesiology University of Virginia Health Sciences Center; Charlottesville, VA
2013- John Mickell Endowed Chair, Division Chief, and Professor of Pediatrics and Anesthesiology, Division of Pediatric Critical Care, Virginia Commonwealth University, Richmond, Virginia.
2015- American Board of Pediatrics, Subsection on Pediatric Critical Care

Honors:

1973 Graduated "With Distinction in All Subjects", Cornell University
1977 Alpha Omega Alpha Medical Honor Society
1977 Graduated "Cum laude" Downstate Medical Center
1980 Pediatric Alumni Award for "Outstanding Pediatric Resident", U. of North Carolina Dept. of Pediatrics
1996 The McLemore Birdsong Award for Excellence in Teaching, U. of Virginia, Dept. of Pediatrics
2002 Award for Clinical Excellence, Dept. of Pediatrics, University of Virginia Health Sciences Center
2015 Housestaff Teaching Award, Dept. of Pediatrics, Virginia Commonwealth University

Federal Government Positions

2004-2009 Chairman of the Collaborative Pediatric Critical Care Research Network (of the NICHD)

C. Contribution to Science

My earliest contribution was demonstrating that variation in practice and over-instrumentation in pediatric critical care was potentially harmful. This has been an important theme in much of my clinical research.

1. **Willson DF**, Jiao JH, Donowitz L, and Henley JO: Invasive Monitoring in Infants with Respiratory Syncytial Virus Infection. J Pediatr 1996; 128:357-62. PMID: 8774504
2. **Willson DF**, Jiao JH: Improved Oxygenation after Stopping Neuromuscular Blockade. Intensive Care Med 1997; 23:214-217. PMID: 9069009
3. **Willson DF**, Horn SD, Hendley JO, et al. The Effect of Practice Variation on Resource Utilization in Infants Hospitalized with Viral Lower Respiratory Illness (VLRI). Pediatrics 2001; 108(4): 851-855. PMID: 11581435

I became interested in the use of surfactant as a consequence of a single patient in whom we administered exogenous surfactant as a rescue therapy. The dramatic improvement in that child led to my clinical interest and the research that I have described in Section A.

In 2004 I was asked to chair the Collaborative Pediatric Critical Care Research Network (CPCCRN) newly funded by the NICHD. This position allowed me to interact and collaborate with many of the recognized experts in my field of pediatric critical care and resulted in a large number of successful investigations, a few of which are cited below.

1. **Willson DF**, Dean JM, Meert KM, et al. The Collaborative Pediatric Critical Care Network: Looking Back and Moving Forward. Pediatr Crit Care Med 2010; 11:1-6. PMID: 19794321
2. Anand KJ, **Willson DF**, Berger J, et al. Tolerance and Withdrawal from Prolonged Opiod Use in Critically Ill Children. Pediatrics 2010; 125: e1208-e1225. PMID: 20403936
3. Zimmerman JJ, Donaldson A, Barker RM, Meert KL, Harrison R, Carcillo JA, Anand KJS, Newth CJL, Berger J, **Willson DF**, Jack R, Nicholson C, Dean JM. Real Time Free Cortisol Quantification Among Critically Ill Children. Pediatric Critical Care Medicine 2011; 12:525-531
4. Carcillo J, Dean JM, Holubkov R, Berger J, Meert KL, Anand KJS, Zimmerman J, Newth CJL, Harrison R, Burr J, **Willson DF**, Nicholson C. The randomized comparative pediatric critical illness stress-induced immune suppression (CRISIS) trial. Pediatric Critical Care Medicine 2012: 13(2):165-73. PMID: 22079954

In 2012 two of my colleagues and I organized the Pediatric Acute Lung Injury Consensus Conference (PALICC). Twenty-seven experts from 10 different countries met over the span of two years to develop consensus on definitions, etiology, severity assessment, treatments, and outcomes of pediatric ARDS. This effort has resulted in the acceptance of 12 manuscripts to date and initiation of several international collaborative research projects. The summary article for the conference is below.

The Pediatric Acute Lung Injury Consensus Group. Pediatric Acute Respiratory Distress Syndrome: Consensus Recommendations from the Pediatric Acute Lung Injury Consensus Conference. Pediatr Crit Care Med 2015. 16:428-439. PMID: 25647235

Most recently I have become interested in the use of antibiotics, particularly as it pertains to ventilator-associated infection in children. I currently have an international point prevalence study examining the incidence, diagnostic features, and outcome. Relevant publications follow:

1. **Willson DF**, Conaway M, Kelly R, Hendley JO. The Lack of Specificity of Tracheal Aspirates in the Diagnosis of Pulmonary Infection in Intubated Children. Pediatr Crit Care Med 2014; 15:299-305. PMID 24614608
2. **Willson DF**, Kirby A, Kicker JS. Respiratory Secretion Analyses in the Evaluation of Ventilator-Associated Pneumonia: A Survey of Current Practice in Pediatric Critical Care. Pediatr Crit Care Med 2014; 15:715-719. PMID: 25068248
3. **Willson DF**, Webster A, Heidemann S, Meert KL; Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Collaborative Pediatric Critical Care Research Network (CPCCRN). Diagnosis and Treatment of Ventilator-Associated Infection: Review of the Critical Illness Stress-Induced Immune Suppression Prevention Trial. Pediatr Crit Care Med 2016 Feb. 17 (Epub ahead of print). PMID: 2689020
4. **Willson DF,** Hoot M, Khemani R, et al. Pediatric Ventilator-associated Infections: The VAIN Study. Pediatr Crit Care Med 2017; 18(1):e24-e34.

D. Research Support

Current Grant Support

Decreasing Antibiotic Exposure in Infants with Suspected Ventilator-Associated Infection. Private Source Private Source Douglas Willson, PI. 2017 -- 2019

Diagnosis, Outcomes, and Microbiome in Pediatric Ventilator-Associated Infection. Virginia Commonweath University Presidential Research Fund. Douglas Willson, PI. 2015-16

Age of Blood in Children: Phil Spinella, PI. Prime Award No.: 1U01HL116383-01. Randomized control trial of usual care vs. transfusion of blood that is less than 7 days old. Sponsored by NHLBI.

Ventilator-associated infections in children (VAIN). Doug Willson, PI. International observational study of incidence, treatment, and outcomes of children in the PICU diagnosed with ventilator associated infection. No financial support.

The Airway Microbiome: Doug Willson, PI. Analysis of the microbiome in sequential tracheal aspirates obtained from intubated patients in the PICU. Sponsored by Private Source Private Source

Past Grants and Contracts

Critical Pertussis in U.S. Children. Carol Nicholson, Primary Investigator. NICHD/Children's National Medical Center.

Calfactant in ARDS due to Direct Lung Injury (Investigator-initiated study). Douglas Willson, PI. Funded by Private Source 2010 – 2013.

Observational Survey of the Management of Anemia in Critically Ill Pediatric Patients. Private Source Private Source Douglas Willson, Co-PI. 2004-2006

BAA Grant National Institutes of Health, National Heart, Lung, and Blood Institute (SPONSOR) under Award Contract Number HHSN268200425210C. Alan Morris, PI. University of Virginia subcontract 2005-2007

Multicenter trial of Calfactant (Infasurf calf lung surfactant extract) in Pediatric Respiratory Failure. This is a 29 center trial that began 4/1/00 funded by Private Source Principle investigator is Doug Willson.

Mechanisms and Physiologic Significance of Renal Urodilatin Secretion. Private Source Private Source

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Kelley M. Dodson, MD

eRA COMMONS USER NAME (credential, e.g., agency login) [eRA Commons User Name]

POSITION TITLE: Associate Professor and Residency Program Director, Otolaryngology/Head and Neck Surgery

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Virginia, Charlottesville VA	BA	06/96	Biology/Spanish
George Washington University, Washington DC	MD	05/00	Medicine
Virginia Commonwealth University Otolaryngology/Head and Neck Surgery Residency Training, Richmond, VA		05/05	Otolaryngology

A. Personal Statement

As an Otolaryngologist/Head and Neck Surgeon specializing in pediatrics, I will contribute to the translational role on the project by identifying clinical data which can be utilized to develop models to assess aerosol delivery efficiency within the sinonasal and respiratory system. As a member of the Virginia Commonwealth University Nasal Interest Research Group, I am currently involved with a multidisciplinary research team dedicated to the study of a variety of diseases which affect the sinonasal and respiratory system. My role in the current project will be to identify pre-existing infant imaging (CT or MR) of the sinonasal and upper airway that can be used to build computational and in vitro models. These image sets will include the entire upper airway from the nostrils through the larynx and trachea. Target models are a pre-term infant (~1600 g) and a full term infant (~ 3550 g). I will also ensure that the nasal geometries constructed are realistic and representative of the infant population at these different development stages. As my collaboration with Dr. Longest and Hindle has previously demonstrated for adults, the combination of an active dry powder inhaler system and nose-to-lung aerosol administration provides an attractive approach for delivering inhaled medications to adult, pediatric and infant patients.

B. Positions and Honors

Employment

2000-01 Internship, Department of General Surgery, Virginia Commonwealth University Medical Center Richmond, VA

2001-05 Residency, Department of Otolaryngology/Head and Neck Surgery, Virginia Commonwealth University Medical Center, Richmond, VA.

2005-2013 Assistant Professor of Otolaryngology/Head and Neck Surgery, Virginia Commonwealth University Medical Center, Richmond VA.

2013- Associate Professor of Otolaryngology/Head and Neck Surgery and Residency Program Director, Virginia Commonwealth University Medical Center, Richmond VA.

Honors and Awards
Certificate of Intermediate Honors, University of Virginia 1994, Graduated with High Distinction, University of Virginia 1996, Alec Horwitz Memorial Award for the highest percentage of honors within the first year of Medical School, 1997, The Goddard Prize in Pharmacology for the highest scholastic standing 1998, The National Pathology Honor Society 1998, the Alec Horwitz Memorial Award for the highest score on Step I of the USMLE 1998, The Huron Lawson Award for the highest scholastic standing in Obstetrics and Gynecology, 2000; The Oscar Benwood Hunter Award for the highest scholastic standing in Pathology, 2000; Alpha Omega Alpha (AOA) Honor Society, 2000; Lawrence Rapee Award for the student with the highest overall scholastic standing 2000; The John Ordonaux Valedictorian Award, 2000. National Institutes of Health Loan Repayment Program Recipient 2006-2009. Richmond Magazine "Top Doc" 2011-present.

Professional Societies
2001-present American Academy of Otolaryngology/Head and Neck Surgery Member
2001-present Virginia Society of Otolaryngology Member
2003-present American Academy of Otolaryngic Allergy Member
2003-present American Society of Human Genetics Member
2006-present Society of University Otolaryngologists Member

C. Contribution to Science

1. Characterization of Pediatric Chronic Rhinosinusitis

I have recently received funding to begin the first characterization of the sinus microbiome in subjects with cystic fibrosis, currently enrolled in a clinical study evaluating the effectiveness of nebulized aztreonam in the management of cystic fibrosis. This study is a unique contribution to the literature as the CF nasal microbiome has not been examined and no longitudinal studies of sinus microbiome in any capacity have been published to date. Currently, I am the PI and co-investigator on two other funded research grants with this group examining the effects of guaifenesin on CRS in children, and the mucus properties associated with acute and allergic rhinitis. All of these projects are in the enrollment phase but are expected to produce a better understanding of the mucus properties in the chronic rhinosinusitis state. Together, we have analyzed middle ear effusions for their rheologic properties and I have also completed prior nasal airway modeling studies with the PI of the current proposal, Dr Longest. We anticipate the results of these projects to fuel a larger expanded multi-center drug study for sinonasal CF, including a larger study of nasal aztreonam as well as other sinonasal aerosols beneficial for CF and non CF related CRS.

Walenga RL, Tian G, Hindle M, Yelverton J, **Dodson K**, Longest PW. Variability in nose-to-lung aerosol delivery. Journal of Aerosol Science 2014;78:11-29.

Dodson KM, Cohen RS, Rubin BK. Fluid Characteristics in Pediatric Otitis Media with Effusion Int J Pediatr Otorhinolaryngol. 2012 Dec;76(12):1806-9.

2. Characterization of Unilateral Hearing Loss

I have been the first to investigate the genetic basis of unilateral hearing loss utilizing a hereditary deafness repository as well as the first to describe the risk factors associated with the development of unilateral hearing loss after failing universal newborn hearing screening. These contributions to the literature have raised awareness of potential familial and genetic contributions in pediatric congenital unilateral hearing loss and have brought to light the risk factor associations statistically more likely to be associated with the development of confirmed unilateral hearing impairment.

Dodson KM, Alexandros Georgolios , Noelle Barr, Bich Nguyen, Aristides Sismanis, Kathleen S Arnos, Virginia W Norris, Derek Chapman, Walter E Nance Arti Pandya "Etiology of Unilateral Hearing Loss in a National Hereditary Deafness Repository" Am J Otolaryngol. 2012 Apr 24.

Yelverton JC, Dominguez LM, Chapman DA, Wang S, Pandya A, **Dodson KM**. Analysis of Risk Factors Associated with Unilateral Hearing Loss Identified Through Newborn Hearing Screening. JAMA Otolaryngol Head Neck Surg. 2013 Jan 1;139(1):59-63.

Dodson KM, Sismanis A, Nance WE. Familial Unilateral Deafness and Delayed Endolymphatic Hydrops. Am J Med Genet A. 2007 May 11;143A(14):1661-1665.

Chapman DA, Stampfel CC, Bodurtha JN, **Dodson KM**, Pandya A, Lynch KB, Kirby RS. The Impact of Co-Occurring Birth Defects on the Timing of Newborn Hearing Screening and Diagnosis.Am J Audiol. 2011 Sep 22.

3. Genetic Hearing Loss

I have worked on a number of congenital and hereditary deafness projects, all further characterizing less well defined types of hearing loss or additional genotype phenotype correlations among more well studied forms of hearing impairment (connexin deafness). Our group was the first to describe the prevalence of vestibular disorders among those with DFNB1 deafness, and have completed vestibular testing on subjects with this condition. I have also characterized the clinical and audiologic features of mitochondrial deafness from a national hereditary deafness repository. We have also advocated for newborn screening for CMV and other forms of genetic deafness to aid with early intervention and habilitation of the hearing impaired.

Dodson KM, Blanton SH, Welch KO, Norris VW, Nuzzo RL, Wegelin JA, Marin RS, Nance WE, Pandya A, Arnos KS. Vestibular dysfunction in DFNB1 deafness. Am J Med Genet A. 2011 May;155A(5):993-1000.

Yelverton JC, Arnos KA, Xia XJ, Nance WE, Pandya A, and **Dodson KM.** The clinical and audiologic features of hearing loss due to mitochondrial mutations. Otolaryngol Head Neck Surg. 2013 Jun;148(6):1017-22.

Nance WE, Lim BG, **Dodson KM**. Importance of Congenital Cytomegalovirus Infections as a Cause for Prelingual Hearing Loss. J Clin Virol. 2006 Feb;35(2):221-5.

D. Research Support

Private Source

PI: Kelley Dodson
Title: Longitudinal Evaluation of the Sinus Microbiome in Cystic Fibrosis.
Objective: To characterize the composition and diversity of microbial communities that colonize the paranasal sinuses of patients with CF, and evaluate their change over time and relationship to clinical variables, including quality of life (QoL), pulmonary function tests (PFTs), and daily symptoms.

NIH/NHLBI R01
Longest (PI), Hindle (PI), Mossi (I), and Dodson (I) 6/1/11 – 3/31/17
Title: Effective Delivery of Pharmaceutical Aerosols during Non-Invasive Ventilation
Objective: The objective of this study is to develop pharmaceutical delivery systems for adults that can significantly enhance aerosol deposition in the lungs during non-invasive ventilation (NIV) using a condensational growth approach.

Private Source

Dodson (PI) 10/31/10 - no cost-extension through 2016
Title: The Effect of Oral Guaifenesin on Pediatric Chronic Rhinitis: A Pilot Study.
Objective: Goals of the project are to determine if guaifenesin is effective in the treatment of chronic rhinosinusitis in children..

Private Source

Rubin (PI) Dodson (I) No Cost extension through 2016
Title: An Observational Study to Evaluate the Relationship of Nasal Mucus Properties and Symptoms in Acute Rhinosinusitis.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 1053004460000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Virginia Commonwealth University

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Philip	W	Longest		PD/PI					20,824.00	7,747.00	28,571.00
2.		Philip	W	Longest		PD/PI					30,852.00	2,345.00	33,197.00
3.		Rebecca	L	Heise		Co-Investigator					12,099.00	920.00	13,019.00
4.		Michael		Hindle		PD/PI					38,580.00	14,352.00	52,932.00
5.		Kelley		Dodson		Co-Investigator					1,851.00	689.00	2,540.00
6.		Douglas	F	Willson		Co-Investigator					1,424.00	530.00	1,954.00
7.		Bruce	K	Rubin		Co-Investigator					1,851.00	689.00	2,540.00

Institutional Base Salary

EFFORT

Total Funds Requested for all Senior Key Persons in the attached file **0.00**

Additional Senior Key Persons: File Name:

Total Senior/Key Person **134,753.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	24	0	0	100,000.00	7,600.00	107,600.00
3	Graduate Students	36	0	0	79,000.00	0.00	79,000.00
	Undergraduate Students						
	Secretarial/Clerical						
5	**Total Number Other Personnel**					**Total Other Personnel**	**186,600.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**321,353.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-16-160 . Received Date: 2017-02-02T16:07:33.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 1053004460000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Virginia Commonwealth University

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		**0.00**
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	9,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**9,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12328492
Funding Opportunity Number: PA-16-160 . Received Date: 2017-02-02T16:07:33.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 1053004460000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Virginia Commonwealth University

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	79,000.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8 . Others	52,566.00
Total Other Direct Costs	**131,566.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**461,919.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research on Campus (MTDC)	55	419,153.00	230,534.00
		Total Indirect Costs	**230,534.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes 301-492-4852

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**692,453.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 1053004460000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Virginia Commonwealth University

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Philip	W	Longest		PD/PI					20,824.00	7,747.00	28,571.00
2.		Philip	W	Longest		PD/PI					30,852.00	2,345.00	33,197.00
3.		Rebecca	L	Heise		Co-Investigator					12,341.00	938.00	13,279.00
4.		Michael		Hindle		PD/PI					39,351.00	14,639.00	53,990.00
5.		Kelley		Dodson		Co-Investigator					1,851.00	689.00	2,540.00
6.		Douglas	F	Willson		Co-Investigator					1,453.00	541.00	1,994.00
7.		Bruce	K	Rubin		Co-Investigator					1,851.00	689.00	2,540.00

Institutional Base Salary

EFFORT

Total Funds Requested for all Senior Key Persons in the attached file **0.00**

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **136,111.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	24	0	0	102,000.00	7,752.00	109,752.00
3	Graduate Students	36	0	0	81,000.00	0.00	81,000.00
	Undergraduate Students						
	Secretarial/Clerical						
5	**Total Number Other Personnel**					**Total Other Personnel**	**190,752.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**326,863.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-16-160 . Received Date: 2017-02-02T16:07:33.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 1053004460000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Virginia Commonwealth University

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period:** 2

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		**0.00**
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	9,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**9,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12328492
Funding Opportunity Number: PA-16-160 . Received Date: 2017-02-02T16:07:33.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 1053004460000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Virginia Commonwealth University

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	72,500.00
2. Publication Costs	1,500.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8 . Others	54,668.00
Total Other Direct Costs	**128,668.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**464,531.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research on Campus (MTDC)	55	420,055.00	231,030.00
		Total Indirect Costs	**231,030.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes 301-492-4852

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**695,561.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 1053004460000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Virginia Commonwealth University

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Philip	W	Longest		PD/PI	Institutional Base Salary	EFFORT			20,824.00	7,747.00	28,571.00
2.		Philip	W	Longest		PD/PI					30,852.00	2,345.00	33,197.00
3.		Rebecca	L	Heise		Co-Investigator					6,804.00	517.00	7,321.00
4.		Michael		Hindle		PD/PI					40,138.00	14,931.00	55,069.00
5.		Douglas	F	Willson		Co-Investigator					1,482.00	551.00	2,033.00
6.		Bruce	K	Rubin		Co-Investigator					16,659.00	6,197.00	22,856.00

Total Funds Requested for all Senior Key Persons in the attached file **0.00**

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **149,047.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	24	0	0	104,040.00	7,908.00	111,948.00
2	Graduate Students	24	0	0	55,000.00	0.00	55,000.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Other Professional	6	0	0	37,500.00	13,950.00	51,450.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**218,398.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**367,445.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-16-160 . Received Date: 2017-02-02T16:07:33.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 1053004460000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Virginia Commonwealth University

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period:** 3

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		**0.00**
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12328492
Funding Opportunity Number: PA-16-160 . Received Date: 2017-02-02T16:07:33.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 1053004460000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Virginia Commonwealth University

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	69,750.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8 . Others	42,271.00
Total Other Direct Costs	**112,021.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**485,466.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research on Campus (MTDC)	55	453,795.00	249,587.00
		Total Indirect Costs	**249,587.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes 301-492-4852

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**735,053.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 1053004460000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Virginia Commonwealth University

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Philip	W	Longest		PD/PI	Institutional Base Salary	EFFORT			20,824.00	7,747.00	28,571.00
2.		Philip	W	Longest		PD/PI					30,852.00	2,345.00	33,197.00
3.		Rebecca	L	Heise		Co-Investigator					6,940.00	527.00	7,467.00
4.		Michael		Hindle		PD/PI					40,941.00	15,230.00	56,171.00
5.		Douglas	F	Willson		Co-Investigator					1,512.00	562.00	2,074.00
6.		Bruce	K	Rubin		Co-Investigator					16,659.00	6,197.00	22,856.00

Total Funds Requested for all Senior Key Persons in the attached file **0.00**

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **150,336.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	24	0	0	106,120.00	8,066.00	114,186.00
2	Graduate Students	24	0	0	56,000.00	0.00	56,000.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Other Professional	6	0	0	37,500.00	13,950.00	51,450.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**221,636.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**371,972.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-16-160 . Received Date: 2017-02-02T16:07:33.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 1053004460000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Virginia Commonwealth University

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 4**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		**0.00**
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 1053004460000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Virginia Commonwealth University

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	75,250.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8 . Others	43,963.00
Total Other Direct Costs	**119,213.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**497,185.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research on Campus (MTDC)	55	464,246.00	255,335.00
		Total Indirect Costs	**255,335.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes 301-492-4852

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**752,520.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Virginia Commonwealth University

A. Senior/Key Person

Dr. Worth Longest (PI, [EFFORT]ACAD/[EFFORT]SUM) will serve as PI for this project. He will have the responsibility to coordinate and oversee all aspects of the project. Dr. Longest is a Professor of Mechanical and Nuclear Engineering and holds a joint appointment in the Department of Pharmaceutics at Virginia Commonwealth University. As PI/PD (under the multiple PI/PD plan), Dr. Longest will be primarily responsible for the computational modeling and validation aspects of the project, as well as developing the new prototyped devices and the production of characteristic airway models for *in vitro* testing. To fulfill the goals of this study, Dr. Longest will supervise a post-doc and graduate student, perform some CFD simulations and validation studies, and be primarily responsible for the development and optimization of the new delivery devices. To facilitate an effective collaboration, Dr. Longest in collaboration with Dr. Hindle will schedule and lead regular meetings of the project participants for the discussion of results, progress, and direction. Dr. Longest will be designated as the NIH contact and responsible person for budgeting and reporting purposes. Additional details are provided in the Multiple PI/PD Leadership Plan.

Dr. Michael Hindle (PI, [EFFORT]CAL) will serve as PI for this project. He will have the responsibility to coordinate and oversee all aspects of the project. He is the Peter R. Byron Distinguished Professor of Pharmaceutics at Virginia Commonwealth University. As PI/PD (under the multiple PI/PD plan), Dr. Hindle will guide and coordinate the formulation development and testing aspects of this study in order to meet the project timeline. He will design, conduct and supervise all aspects of the powder formulation developments, together with the design of experiments to measure in vitro delivery, deposition and condensational growth in the realistic airway geometries using the newly developed devices described in this proposal. To facilitate an effective collaboration, Drs. Hindle and Longest will lead regular meetings of the project participants for the discussion of results, progress, and direction. Dr. Hindle will be responsible for supervising the work of a post doctoral fellow (4 years) and a graduate student (4 years).

Dr. Rebecca Heise (Investigator, [EFFORT]SUM Y1 and Y2; [EFFORT]SUM Y3 and Y4) is an assistant professor of Biomedical Engineering in the VCU School of Engineering. Dr. Heise specializes in lung injury, inflammatory pulmonary markers, and animal lung models. Dr. Heise will test the *in vivo* efficacy of the deposited surfactant aerosol particles in an animal model of surfactant depletion.

Dr. Kelley Dodson (Investigator, [EFFORT] CAL) will assist the PIs in Year 1 and Year 2. Dr. Dodson is an Associate Professor and Residency Program Director of Pediatric Otolaryngology, Congenital and Genetic Hearing Loss, Otology, and General Otolaryngology. Her role in the current project is to identify pre-existing imaging of the nasal and upper airway that can be used to build computational and *in vitro* infant airway models. Once these geometries are complete, she will also verify their anatomical accuracy.

Dr. Douglas Willson (Investigator, [EFFORT]CAL) is Professor and Chief of Pediatric Critical Care Medicine at VCU. Dr. Willson has worked with surfactant replacement therapy in critical care medicine for over a decade. He has published several seminal papers on the subject and led large-scale clinical trials on the use of surfactant replacement therapy. In this project, Dr. Willson will provide clinical insights in the delivery of surfactant replacement therapy to infants and advise the PIs on practical aspects of implementing the newly developed delivery devices in patients.

Dr. Bruce Rubin (Investigator, [EFFORT]CAL Y1 and Y2; [EFFORT] CAL Y3 and Y4) is the Jessie Ball duPont Distinguished Professor and Chair of the Department of Pediatrics at the VCU Medical Center and has appointments as Professor of Biomedical Engineering and an Adjunct Professor of Physiology and Biophysics at Virginia Commonwealth University. Dr. Rubin is a pediatric pulmonologist, an expert in airway disease, and a clinical aerosol scientist. He has extensive experience in pediatric respiratory aerosol delivery and respiratory biomaterials. Dr. Rubin's lab will test the *in vivo* surfactant aerosol efficacy in an infant-size ferret model.

B. Other Personnel

Postdoctoral Researcher, TBD (Mechanical Engineering) (12.0 CAL) will be supported (salary and health insurance) on this project and will assist Dr. Longest with the airway CFD simulations and airway prototyping. This will include developing airway models, producing CFD meshes and physical models of the airway geometries, and simulation of aerosol transport and deposition. The post-doc will closely collaborate with the VCU Department of Pharmaceutics to ensure that the *in vitro* models are appropriate for regional aerosol deposition testing. He or she will be responsible for validating model simulations with experiments provided by Pharmaceutics. The postdoc will closely collaborate with Dr. Longest and the engineering graduate student to develop optimized delivery devices and strategies. He or she will be responsible for producing written manuscripts and making presentations to document study findings. Dr. Longest will be responsible for supervising the postdoc's effort on this project.

Postdoctoral Researcher, TBD (Pharmaceutics) (12.0 CAL). This postdoc will assist Dr. Hindle in the production of combination EEG powder formulations. He or she will perform the formulation and device optimization studies and then assess in vitro device and airway deposition using the aerosol delivery devices and noninvasive ventilation systems. In addition, the interdisciplinary nature of the project will require frequent interaction with faculty and postdocs in the Dept. of Mechanical Engineering for cross-fertilization of ideas. Dr. Hindle will be responsible for supervising the postdoc's efforts throughout the project.

Graduate Student, TBD (Mechanical Engineering) (12.0 CAL) will be supported (salary and tuition) on this project and will be primarily responsible for assisting Dr. Longest in the development of the new infant respiratory drug delivery system as described in the proposal. This will include CFD simulations of device performance, optimization of the design, rapid prototyping, experimental testing, and device revisions. The developed prototypes will be initially tested in the engineering lab for performance in terms of pressure drop and flowrate as well as rapid screening of aerosolization characteristics. The student will develop expertise in using both CFD simulations and *in vitro* testing for rapid device design and analysis. Dr. Longest will directly assist the graduate student to ensure that the devices are developed and tested in a timely manner, which is critical for the advancement of the project.

Graduate Student, TBD (Pharmaceutics) (12.0 CAL). This student will be primarily responsible for assisting Dr. Hindle in the extensive analytical component of these studies. The student will be responsible for the development HPLC-MSMS analytical methods for the profiling of the complex surfactant phospholipid and protein mixtures. In addition, he/she will assist in the development of quantitative methods for use during the formulation characterization studies, the stability studies, the in vitro aerosol deposition studies, and the device testing studies. The graduate student will be responsible for the maintenance and operation of the HPLC-MSMS.

Graduate Student, TBD (Biomedical Engineering) (12.0 CAL in Y1 and Y2). This student will assist Dr. Heise in conducting the animal experiments in a rat surfactant depletion model. These experiments will include inducing lung injury in the rats through surfactant depletion and delivery of the surfactant formulations. The graduate student will be responsible for performing all experiments and analyzing the data with Dr. Heise.

Assistant Professor, TBD (Pediatrics) (6.0 CAL in Y3 and Y4). The assistant professor will be an expert in airway disease and a research manager for Dr. Rubin. He/she will be responsible for conducting the majority of the proposed studies under Dr. Rubin's direct supervision. He/she will maintain IACUC regulatory requirements, handle ordering and perform the ferret airway procedures and histology analysis.

C. Equipment – none requested.

D. Travel

Travel funds for the personnel in the Departments of Pharmaceutics and Mechanical Engineering in the amount of $3,000 for each department are requested annually. Travel funds for the personnel in the Department of Biomedical Engineering in the amount of $3,000 are requested in Y1 and Y2. These funds will be used to support travel for the respective faculty in each department, post docs and graduate students to domestic conferences, universities, and agencies to present the results of the project. Estimated costs include transportation costs, per diem, conference registration, and the cost of preparing material for presentation.

E. Participant/Trainee Support Costs – none requested.

F. Other Direct Costs

1. Materials and Supplies

In Years 1 and 2, when the rat screening tests are conducted, funds in the amount of $79,000 and $72,500 are requested, respectively. In Years 3 and 4, during the ferret animal model efficacy testing, funds in the amounts of $69,750 and $75,250 are requested, respectively.

Materials (Mechanical Engineering)

Computational supplies ($10,000 per year). Renewals of software licenses for Fluent, Mimics, Tecplot, and Ensight are requested each year. This software is needed to build the computational meshes, perform the CFD simulations, and post-process the results.

Rapid prototyper resin and maintenance agreement ($4,000 per year). The purchase of necessary resin for the in-house rapid prototyper that will be used to construct the respiratory airway models and devices is requested each year, together with funds for maintenance on this essential equipment item.

Fabrication and testing of prototype devices ($6,000 Y1 and Y2; $1,000 Y3 and Y4). Materials and supplies for fabricating and testing the device prototypes including materials, shop expenses, filters, consumables, and cleaning supplies are requested.

Multiprocessor workstation. Funds are requested in Year 1 & 2 ($9,000 total) to purchase two dedicated multiprocessor workstations. The workstations will have 2 processors with four computational cores, a total of 64+ GB of RAM, approximately 1 TB of storage capacity, and full backup capabilities. Purchase of these dedicated machines is necessary for the newly hired postdoc and graduate student to have the computational power in-house to allow for efficient simulation of the proposed respiratory aerosol dynamics, post-processing of the results, and long-term storage of the findings.

Materials (Pharmaceutics)

HPLC-MSMS analytical supplies ($10,000 per year), as described above, drug quantification by HPLC-MSMS is an essential means of characterizing the performance of the developed formulations, assessing their stability and characterizing their aerosol performance. Funds are requested for operation an HPLC-MSMS, which requires HPLC solvents ($3000 per year), columns ($1500 per year), equipment maintenance ($3500), tubing, vials, connectors, filters, cleanup, and waste disposal ($2000 per year).

Physico-chemical characterization supplies ($2000 per year in Years 1 and 2), these costs cover SEM disposable supplies, together with DSC pans, Karl Fisher reagents required to fully characterize the generated combination formulations.

General aerosol laboratory supplies and chemicals ($5000 per year plus $3000 in Y2), these include cascade impaction substrates, gloves, safety supplies including disposable mask filters, tubing, filters, vacuum pump

supplies and wash solvents. Included in this request is a one-time replacement of spray nozzles for the Buchi spray dryer, which will be required to ensure optimal spray drying performance after the 1st year of use ($3000 in Y2).

Drug and excipient raw materials ($23,000 in Y1, $20,000 in Y2 and $15,000 in Y3 and Y4), the major expense will be a recurring cost of purchasing of commercial infant surfactant solutions (Survanta, Curosurf and Surfaxin ~ $600 per bottle) to be used in the spray dried EEG formulation development. Each bottle produces approximately 800 mg of EEG formulation. We plan to purchase 30 bottles of surfactant per year in Y1 and Y2, and based on preliminary studies, this should provided sufficient surfactant for the formulations optimization in Y1 and Y2, together with the production and evaluation of the optimized formulation in Y3 and Y4 (20 bottles per year). Additional funds are requested in Y1 for purchase of other EEG excipients, phospholipid and protein standards, including bulk purchases of mannitol ($250 per 500g), leucine ($250 per 250g) and DPPC ($1250 per 10g). Funds for Surfactant Protein B and Surfactant Protein C ($1250) are also requested.

Materials (Biomedical Engineering)

Male and female Sprague-Dawley rats will be purchased from Charles River (120 rats + housing, $3,000 in Y1 and $3,000 in Y2).

Histological processing of rat lungs ($2,000 in Y1 and $2,000 in Y2). Supplies will be used for service fees and staining reagents.
Flexivent and Blood Gas Machine Consumables and Maintenance. Animal surgery consumables, including instruments and tubing, and maintenance on the devices, including small part replacement and reagents, are requested ($3,000 in Y1 and $3,000 in Y2).

Computer: Funds ($2,000) are requested in Y1 for a laptop workstation to run the Flexivent mechanical ventilation system.

Funds for a Cytospin ($4,500) are requested in Y1 for the device that will allow for centrifugation of rat lavage fluid for inflammatory cell analysis.

Materials (Pediatrics)

Male ferrets will be purchased from Charles River (20 Ferrets including shipping and housing, $18,000 in Y3 and $22,000 in Y4).

Consumables ($4,950 Y3 and $6,050 Y4) to cover the cost of surgical supplies, gloves and reagents.

Histological processing of ferret lungs and airways ($1,800 in Y3 and $2,200 in Y4). Supplies will be used for service fees for paraffin blocking, slicing and staining reagents.

2. **Publication Costs** – (Biomedical Engineering $1500 in Y2) for publication page charges and open access fees
3. **Consultant Services** – none requested.
4. **ADP/Computer Services** – none requested.
5. **Subawards/Consortium/Contractual Costs** – none requested.
6. **Equipment or Facility Rental/User Fees** – none requested.
7. **Alterations and Renovations** – none requested.
8. **Graduate Student Tuition**

Tuition remission in lieu of part of wages for the 3 graduate student researchers is requested.

9. **Post-Doctoral Health Insurance**

Funds in the amount of $9,800 per year are requested with a 4% increase in Y2, Y3 and Y4 for 2 Post Doctoral researchers.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		570,247.00
Section B, Other Personnel		817,386.00
Total Number Other Personnel	20	
Total Salary, Wages and Fringe Benefits (A+B)		1,387,633.00
Section C, Equipment		0.00
Section D, Travel		30,000.00
1. Domestic	30,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		491,468.00
1. Materials and Supplies	296,500.00	
2. Publication Costs	1,500.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	193,468.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		1,909,101.00
Section H, Indirect Costs		966,486.00
Section I, Total Direct and Indirect Costs (G + H)		2,875,587.00
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	
Research Plan Section	
2. Specific Aims	SpecificAims.pdf
3. Research Strategy*	Research_Plan_final.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	Protection_of_Human_Subjects.pdf
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	Vertebrate_Animal_Care_Plan.pdf
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	Leadership_Plan.pdf
12. Consortium/Contractual Arrangements	
13. Letters of Support	
14. Resource Sharing Plan(s)	Resource_Sharing.pdf
15. Authentication of Key Biological and/or Chemical Resources	Authentication_Plan.pdf
Appendix	
16. Appendix	

SPECIFIC AIMS

Surfactant replacement therapy is currently one of the most effective treatments for infants suffering from respiratory distress syndrome (RDS) [1, 2] and decreases chronic lung disease (CLD) [3, 4]. The only delivery method that is currently approved for pulmonary surfactants is instillation through an endotracheal tube (ETT) as a liquid bolus (~4 ml/kg or greater), followed by a period of hand bagging or mechanical ventilation [5-7]. Due to instillation of the large liquid bolus, side effects can include hypoxia, hypotension, fluctuations in hemodynamics and cerebral perfusion, and increased risks of pulmonary and intracranial hemorrhage [5, 8, 9]. Intubation with an ETT or delivery catheter and invasive mechanical ventilation also carry well documented risks including lung injury and infection [3, 9].

The use of aerosolized surfactant has been proposed as a method to avoid liquid instillation [5-7, 10]. Primary limitations of aerosolized surfactants have been very low lung delivery efficiencies (typically ~1%) [11], long delivery times (~3 hours with mesh nebulizers) [12], and poor distribution to the alveolar region. Nevertheless, studies that have successfully delivered aerosolized surfactant to the lungs have demonstrated improved symptoms of RDS and improved lung function with reduced inflammation compared to surfactant instillation [13-15]. A significant advantage of aerosolized surfactants is the ability to avoid intubation and the associated risks of invasive ventilation (lung injury and infection) in cases where noninvasive ventilation (NIV) is the preferred method of ventilatory support.

The **goal** of this study is to develop formulations and devices for the effective delivery of aerosolized surfactants to the lungs of infants using the nose-to-lung (N2L) route thereby avoiding intubation. To achieve high efficiency lung delivery, the excipient enhanced growth (EEG) approach [16-18] will be implemented in which primary **submicrometer** particles are formed through spray drying [19] and contain the surfactants and a **hygroscopic excipient**. The initial small size of the aerosolized particles allows for effective penetration through the NIV system and infant upper airways [20]. Inclusion of the hygroscopic excipient in the primary particles fosters aerosol size increase inside the lungs and effective deposition in the alveolar region [21]. The aerosols will be formed with an active inline low-flow dry powder inhaler (DPI) system that implements a three-dimensional **(3D) rod array** [22]. The 3D rod array structure was recently shown to provide high efficiency deaggregation of carrier-free EEG formulations and produced fine particle fractions (FPFs) higher than any other tested device **(FPF > 90%)** [22-24]. A new N2L aerosol delivery device will be developed that can **rapidly and efficiently** administer high doses of the EEG surfactant aerosol and control respiration. Nasal interfaces will be optimized using a streamlining approach [25, 26]. Efficacy of the newly created EEG surfactant formulations will first be screened in a rodent (rat) surfactant depletion model leading to the selection of a primary formulation. Concurrent realistic *in vitro* experiments and complete-airway computational fluid dynamics (CFD) simulations will be used to optimize the lung delivery device and strategy for preterm and term infants. The N2L delivery device, strategy, and efficacy of the EEG formulation will then be evaluated in an **infant-size ferret model**. To develop this therapeutic approach, the following Specific Aims are proposed:

Specific Aim 1. Develop an excipient enhanced growth (EEG) formulation of a lung surfactant that can be efficiently aerosolized, increase in aerodynamic size within the airways, and maintain surfactant function.

Specific Aim 2. Develop and optimize a device for generating and administering surfactant aerosols to infants using the noninvasive nose-to-lung (N2L) route and achieving high efficiency lung delivery.

Specific Aim 3. Adapt the N2L aerosol delivery device and test EEG surfactant aerosol efficacy in an infant-size ferret model compared with surfactant instillation in terms of oxygenation, lung distribution and histology.

Outcomes and Impact. The newly developed aerosolized surfactant system will achieve a lung delivery efficiency of 60% or greater in a highly realistic preterm infant model (and 70% or greater in a full term infant model) with most of the lung dose (>70%) deposited in the alveolar region at a rate of 10 mg of phospholipids in <30 seconds. Successful delivery of aerosolized surfactant will avoid the side effects associated with instillation in compromised infant airways. Efficient N2L delivery will allow for expanded use of NIV respiratory support techniques, such as continuous positive airway pressure (CPAP), thereby avoiding the greater risks associated with intubation and invasive ventilation in many cases. Efficacy testing in animal models will ensure that surfactant function can be maintained following spray drying of surfactant EEG particles. Aerosol delivery and alveolar deposition are expected to provide reduced airway inflammation and edema compared with liquid bolus instillation. In addition to the treatment of RDS, improved surfactant delivery to the alveolar region using the developed EEG surfactant formulation may also improve envisioned surfactant-based therapies of other lung conditions including acute respiratory distress syndrome (ARDS) and mucus retention.

RESEARCH STRATEGY

A. SIGNIFICANCE

A.1 Current Use of Exogenous Surfactants in Infants

The use of surfactant replacement therapy is highly effective for treating infants suffering from RDS [1, 2]. Surfactant deficiency due to prematurity or surfactant inhibition combined with mechanical ventilation initiates a cascade of responses including inflammation, atelectasis, and lung injury leading to the long-term condition of CLD (also referred to as bronchopulmonary dysplasia) [3]. Application of animal derived or synthetic surfactants reduces surface tension in the acinar region making the lungs more compliant, which reduces the work of breathing, decreases the pressure required for mechanical ventilation, and helps prevent cyclic airway collapse and reopening [3, 5]. It is envisioned that surfactant replacement therapy may also be used to effectively treat meconium aspiration syndrome (MAS), ARDS, and mucus retention [5, 6]. However, the use of surfactants to treat these conditions in infants and adults is less successful compared with infant RDS due to complexity of the initiating lung injury, inadequate surfactants, and inadequate delivery [5].

A.2 Limitations of Current Surfactant Administration

The current standard of care administration technique for exogenous surfactants is intubation followed by liquid bolus instillation through a second catheter, which is then frequently followed by a period of mechanical ventilation to provide respiratory support [5-7]. The surfactant is instilled as a suspension in a relatively **large volume** of carrier fluid (~ 4 ml/kg liquid bolus) to help distribute the dose [5, 6, 27]. Liquid blockage of the ETT or airways, which occurs with surfactant instillation, is associated with hypoxia and hypotension [5]. Potential side effects of surfactant instillation include fluctuations in cerebral perfusion [8, 9, 28, 29] and increased risks of pulmonary and intracranial hemorrhage [30]. Delivery by instillation requires intubation, which is sometimes unsuccessful [31], and can cause hypoxemia, bradycardia, increased cranial pressure, and trauma [32]. Instillation of the liquid typically requires at least 5 minutes with the surfactant administered in 1/4 doses and cycles of ventilation after each 1/4 dose [33]. After instillation, the subsequent use of mechanical ventilation is a cause of inflammatory lung injury [34], as well as volutrauma and barotrauma leading to CLD [3, 35, 36].

A.3 Aerosolized Surfactants and Non-Invasive Ventilation (NIV)

The delivery of aerosolized surfactants together with noninvasive ventilation (NIV) has been envisioned as a method to reduce the risks associated with instillation and frequent subsequent invasive mechanical ventilation [5-7, 37]. The use of NIV in infants is preferred whenever possible in order to reduce the risks of intubation and airway damage associated with invasive mechanical ventilation [9, 38, 39]. Delivery of aerosols before or during NIV prevents the need for intubation, can potentially reduce surfactant dose, and better targets delivery to the alveolar region. However, primary challenges to effective aerosolized surfactant therapy have been inefficiency in alveolar delivery and long delivery times. Aerosol delivery efficiency to infants on any form of ventilation is known to be low for all types of aerosol generators including MDIs (~1%), jet nebulizers (~1%), and new mesh nebulizers (~1-10% with water soluble drugs) [40-46]. Recent studies by Berggren et al. [11] and Finer et al. [37] implemented vibrating mesh nebulizers for surfactant aerosol delivery and reported 3 hour nebulization times per treatment with approximately 1-6% delivery efficiency out of the device. Despite these delivery challenges, animal studies that have administered a sufficiently small and well-controlled surfactant aerosol have demonstrated improved outcomes. For example, Ellyett et al. [13] demonstrated improved survival in preterm neonatal rabbits as aerosol mass median aerodynamic diameter (MMAD) was reduced from 2.40 µm to 0.44 µm. Studies by Sun et al. [14] and Ruppert et al. [15] also carefully controlled aerosol size and produced surfactant aerosols with MMADs < 2.0 µm. These studies evaluated animal models of lung injury and demonstrated that the small particle surfactant aerosols were capable of reducing inflammation and respiratory resistance, as well as restoring oxygen levels and improving survival.

A.4 Improved Delivery of Aerosolized Surfactant

While the delivery of aerosolized surfactant holds significant potential, successful human delivery has not currently been achieved [5, 6]. Primary themes limiting effective aerosol surfactant treatment are poor lung delivery efficiency and inadequate distribution of the therapy in the lungs. The **goal** of this study is to develop formulations and devices for the effective delivery of aerosolized surfactant to the lungs of infants using the nose-to-lung (N2L) route thereby avoiding intubation. It is proposed that effective N2L aerosol delivery in an infant can be achieved using newly developed condensational growth techniques [16, 18] together with well designed delivery devices [26]. A stable dry powder surfactant formulation will be used based on recent work with excipient enhanced growth (EEG) aerosols [19, 47], and deaggregation of the powder will be achieved with newly developed 3D rod array active dry powder inhaler (DPI) systems [22-24]. Improved alveolar delivery

of surfactant aerosols will allow for more accurate assessments of surfactant performance. The developed delivery technology has the potential to improve the treatment of RDS by allowing for aerosolized surfactant delivery without the need to intubate. Improved surfactant delivery to the alveolar region may also improve surfactant-based treatments of additional lung conditions including MAS, ARDS, and secretion retention.

A.5 Clinical Impact of Aerosolized Surfactants

Instilled liquid surfactants are currently approved for treating RDS in preterm infants, which represents an annual US patient population of approximately 50,000 [48, 49]. Approximately 50% of all RDS cases can be supported with NIV [9, 48], resulting in 25,000 preterm infants with RDS on NIV who could potentially receive surfactants. Based on the data of Hibbard et al. [48], it is reasonable to assume that all of these NIV infants could receive surfactants for RDS. For these infants, intubation is required only for surfactant administration and intubation alone is associated with adverse events in 22% [50] of cases (or an estimated 5,500 occurrences for surfactant administration) with 3.7% [50] of cases being severe (estimated 1,000 occurrences). Inhaled surfactant delivery during NIV could avoid these adverse events in cases where the patient can be support by NIV. Furthermore, intubation alone is associated with **oxygen desaturation in 51.1%** of NICU procedures. Considering instillation of the surfactant liquid after intubation, additional side effects include transient bradycardia (12% for each delivery event) and oxygen desaturation (9.8%), which may significantly delay surfactant administration [33]. Rapid fluctuations of cerebral hemodynamics are also connected with instillation [8]. The clinical impact of these rapid changes in cerebral blood flow is not known [9]; however, intracranial hemorrhage is common in this population [33].

In comparison with developed RDS, aerosol surfactants can be delivered as a prophylactic without the need to intubate. The current preterm annual population in the US is approximately 400,000 infants [48]. Kattwinkel et al. [51] showed that prophylactic surfactant administration by instillation could reduce moderate RDS developed from 12% to 7% in infants between 29 and 32 weeks. Aerosol surfactant delivery would motivate prophylactic use by **removing the need to intubate** and **may improve surfactant efficacy** based on reduced lung edema and inflammation, as reported in recent animal studies [52, 53].

B. INNOVATION

B.1 Condensational Growth with Applications to NIV Aerosol Delivery

A recently proposed set of methods to improve the lung delivery of pharmaceutical aerosols is the controlled condensational growth technique developed by our research group at VCU [18, 54-56]. In this approach, an aerosol is delivered to the respiratory tract with an initial submicrometer or sufficiently small size to minimize device and upper airway deposition. Droplet size increase through condensational growth allows for retention of the aerosol, which without growth would be exhaled. Techniques to produce the required size increase include enhanced condensational growth (ECG) and excipient enhanced growth (EEG). In the ECG approach, the aerosol is delivered with air saturated with water vapor a few degrees above body temperature, which creates supersaturated relative humidity conditions in the lungs to foster condensational growth of the droplets [54, 57]. With EEG, formulated particles contain a combination of a drug and a hygroscopic excipient, and the natural relative humidity in the lungs provides the water vapor source for aerosol size increase [18, 55]. Combination particles that contain both the therapeutic agent and a hygroscopic excipient in order to generate aerosol size increase in the airways are referred to as an EEG formulation [18, 19].

The new approach of controlled condensational growth has been successful at improving lung delivery for orally-administered aerosols [19, 21, 47, 54, 57] and for nose-to-lung delivery with nebulizer generated aerosols [58, 59]. Considering nose-to-lung delivery with a nebulizer, our group [59] previously demonstrated that both EEG and ECG approaches **reduced cannula and nasal depositional losses by an order of magnitude** and delivered approximately **80%** of the loaded dose **to the lungs** with steady flow. Using an aerosol mixer-heater system [60] combined with a mesh nebulizer, our group [61] demonstrated that **synchronizing the aerosol delivery with patient breathing** was important to achieve high efficiency aerosol delivery (>70%) past the nose and to the lung in an adult high flow nasal cannula (HFNC) system. We [62] recently reported computational fluid dynamic (CFD) simulations of nose-to-lung administered EEG aerosols through the conducting tracheobronchial (TB) airways and found minimal nasal depositional loss, droplet growth, and a significant dose enhancement to the lower TB region of 40x compared with marketed inhalers.

Considering DPIs, our group has developed high efficiency devices for oral inhalation of EEG aerosols [24, 47, 63]. Submicrometer combination particles are produced by spray drying [19] and the DPI includes a novel 3D rod array [23] that was shown to best deaggregate EEG formulations [23, 47]. These new formulation and inhaler combinations achieved emitted doses (ED) >75%, fine particle fraction (FPF) <5 μm values >90%,

and initial MMADs ≤1.5 µm, which result in mouth-throat depositional losses <5%. To allow for dry powder aerosol delivery during mechanical ventilation, the EEG formulation and 3D rod array concepts were incorporated in a ventilation bag actuated inline DPI [22]. The new inline DPI device achieved an ED of 80% with FPFs of 85% and a MMAD of 1.5 µm at a flow of 15 LPM, which were target conditions for effectively delivering an EEG aerosol during HFNC in adults. As with other condensational growth applications, the small initial size of the aerosol provides for effective transport through the delivery system and extrathoracic airways [47, 54, 59] with substantial growth and deposition in the lungs [21, 55]. **While submicrometer initial particle size is often targeted, sizes ≤ 1.5 µm frequently provide sufficiently high lung delivery efficiency and increase the final droplet size that can be achieved through enhanced condensational growth.**

B.2 Streamlining of Ventilation Components

Based on high drug depositional losses, current ventilator tubing connectors and patient interfaces used with mechanical ventilation **are not designed for effective aerosol drug delivery**. Previously, Longest et al. [26, 64] proposed a streamlining design approach for ventilator circuit components that eliminates sudden expansions and contractions in the flow path and applies a radius of curvature to changes in flow direction. The streamlined component approach results in significantly more uniform flow with less flow separation, detachment, recirculation, vorticity, turbulence, and decreased aerosol deposition by dispersion and inertial impaction [26, 64]. We [26] demonstrated that the streamlining approach could reduce drug deposition within individual components of a HFNC system by factors as large as 4x. Application of the streamlining approach to the infant wye-connector used in invasive mechanical ventilation reduced drug depositional loss by a factor of 9x [64]; however, this design approach has not yet been applied to infants.

B.3 Dry Powder Aerosols and Surfactants

Dry powder aerosol formulations provide a number of advantages compared with other formulations including product stability [65], high drug mass per inhalation [66], less susceptibility to microbial growth, and low cost [67]. For aerosolized surfactants, dry powder aerosols can be used to reduce the current single-dose delivery time of 3 hours in humans required with mesh nebulizers [11, 12]. Nebulization times of 3 hours may be too long for the surfactant to achieve sufficient concentrations for adsorption and formation of an effective monolayer. In contrast, aerosol doses using current inefficient dry powder systems may be as high as 800 mg/min [15], with very high delivery system and upper airway losses. The few studies that have considered the biological effectiveness of dry powder surfactants report improved respiratory function. Morley et al. [10] reported improved survival of infants (100%) with dry powder surfactant aerosol delivered with a manual resuscitation bag in a small proof of concept study in humans. Ruppert et al. [15] demonstrated significantly improved respiratory conditions using a dry powder aerosol surfactant in rabbit models of lung injury. Furthermore, the study of Ruppert [15] demonstrated that micronization and aerosolization of the dry powder surfactant at high shear did not degrade the surface tension lowering properties of the surfactant.

B.4 New EEG Surfactant Formulation and Delivery System for Infants

A new EEG surfactant powder formulation, produced from marketed surfactant products, will be developed with efficient delivery systems for administering aerosols to infants using the N2L route. The EEG formulation will contain a dispersion enhancer and hygroscopic growth excipient [19] to improve aerosolization and wet the surfactant particles with water, increasing the aerosol MMAD once inside the lungs to improve lung deposition. Previously developed inline DPIs using 3D rod array technology [22] will be advanced to effectively aerosolize the EEG formulation at flow rates and volumes consistent with infant respiration. A new N2L aerosol delivery device will be developed [26, 64], based on guidance from CFD simulations [68], to allow for improved respiratory drug delivery. By combining the EEG delivery approach with the N2L aerosol delivery device, lung delivery efficiencies are expected to increase from current values of 1-10% to the range of 60-70%, as achieved with our previous work in adults [22, 59, 61, 62]. Use of a dry powder formulation will allow for rapid surfactant administration and inclusion of the hygroscopic excipient will enhance alveolar deposition. The delivery system will be developed using a combination of CFD simulations, 3D printing and *in vitro* testing in realistic upper airway geometries. Efficacy of the spray dried surfactant formulation compared with the current standard-of-care (liquid bolus instillation) will be tested in surfactant depletion animal models. It is expected that the new aerosol surfactant formulation will provide an equivalent or superior **improvement in respiratory function with less lung inflammation** compared with liquid bolus instillation [52, 53].

C. APPROACH

The device concepts described below contain proprietary information that Virginia Commonwealth University requests not be released to persons outside the Government, except for purposes of review and evaluation.

C.1 Specific Aim 1: Develop an excipient enhanced growth (EEG) formulation of a lung surfactant that can be efficiently aerosolized, increase in aerodynamic size within the airways, and maintain surfactant function.

Preliminary Data: *Generation of Lung Surfactant EEG Formulations using Spray Drying.* Previous studies have established optimized spray drying conditions to produce submicrometer EEG formulations for a range of pharmaceuticals including spray dried albuterol sulfate, azithromycin, tobramycin and ciprofloxacin hydrochloride [19, 69, 70]. The Buchi Nano (B-90) spray dryer was used based on its capability to produce submicrometer particles by a vibrating mesh mechanism. Spray drying process parameters that have been investigated for EEG formulations included dryer length, spray mesh size and dryer temperature [19]. Other investigators have used high concentration organic solvent stock solutions to prepare spray dried formulations of lung surfactant; however, this approach may affect the integrity and surface activity of these complex lipid and protein mixtures [71, 72]. In our preliminary spray drying studies, we have used a mixed aqueous / organic spray drying solvent to enable the incorporation of the EEG excipients together with marketed lung surfactant formulations containing both phospholipids **and surfactant proteins**. A spray drying stock dispersion was prepared containing 2 ml Survanta (50 mg of total phospholipids) with 50 mg of mannitol (MN) or sodium chloride (NaCl), as the hygroscopic excipient, and 25 mg of L-leucine, as the dispersion enhancer, in 100 ml of water:ethanol (80:20%v/v). A similar approach has also been employed to spray dry a Curosurf-MN-EEG formulation using 0.66 ml of Curosurf (50 mg of total phospholipids). The spray drying conditions were a drying temperature of 70 °C and outlet temperature of 38 °C, with a gas flow of 120 L/min. The stock dispersion was maintained at 5 °C. Free flowing powders were obtained, with high powder yields. Figure 1 shows a SEM image of the Survanta-NaCl EEG formulation with primary particles that were submicrometer in size. To test initial aerosolization characteristics, 10 mg of the Survanta-MN-EEG formulation was loaded into our most developed dry powder inhaler [24] and operated at an adult flow rate into a Next Generation Impactor (NGI). The MMAD of the Survanta-MN-EEG formulation was 1.8 (0.2) µm and was similar to previously generated spray dried EEG formulations indicating comparable and efficient aerosolization of the surfactant formulation.

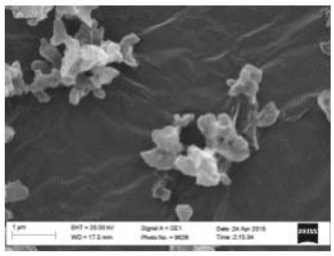

Fig. 1. SEM of spray dried Survanta-NaCl-EEG formulation.

Analytical Method Development for DPPC and Surfactant Proteins. In order to analytically characterize the marketed surfactant formulations and the spray dried EEG formulations, LC-MS methods have been developed for the quantification of phospholipids and surfactant proteins. In these preliminary studies, we have selected DPPC as a quantitative marker to assess its composition in the marketed and spray dried EEG formulations. A simple isocratic method with a HILIC silica column was used to quantify DPPC using mass spectrometry detection of the parent ion in selective ion monitoring mode for $M[H^+]$ = 735. DPPC has a retention time of 2 min using this method. A calibration curve was prepared using standard concentrations of DPPC and was used to estimate the content of DPPC in the Survanta formulation and in the EEG spray-dried formulations. For the analysis of the surfactant protein and phospholipid mixtures, an LC-MS method has been developed using a BEH C4 protein column which enables separation of the surfactant proteins (SP-B and C) from DPPC and the other phospholipids without the need for delipidation and is based on the method of Gustafsson et al. [73]. Figure 2 shows ion chromatograms for bovine SP-C (top) with a retention time of 6 min. and DPPC (bottom) with a retention time of 13 min.

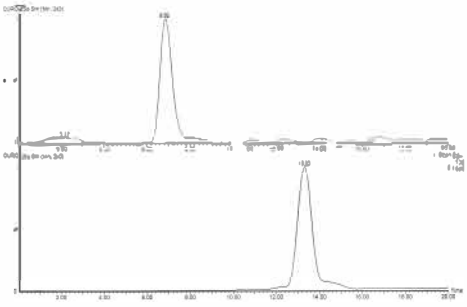

Fig. 2. Ion chromatograms of bovine **surfactant protein C** (top) and **DPPC** (bottom).

Coupled In vitro and In silico Methods to Evaluate Droplet Growth as a Function of Inhalation Time. A series of studies have been published by our research group to investigate inhalation time vs. size increase of EEG aerosols [17, 18, 55]. In these studies we developed a series of mathematical and CFD models to simulate EEG aerosol droplet size vs. time of flight in the lungs. We validated these models with extensive comparisons to *in vitro* data [16, 17, 55, 56]. In an infant, inspiration and full respiratory cycles require approximately 0.5 s and 1.5 s, respectively [74]. Predictions with a validated numerical model [55] indicate that 0.5 s within the humid airways produces a droplet diameter greater than 3 µm for 50:50 combination surfactant:MN particles with an initial particle diameter (d_o) of 1.5 µm (Fig. 3). Additional size increase of the droplet is observed to occur during the full 1.5 s period. At 0.5 s,

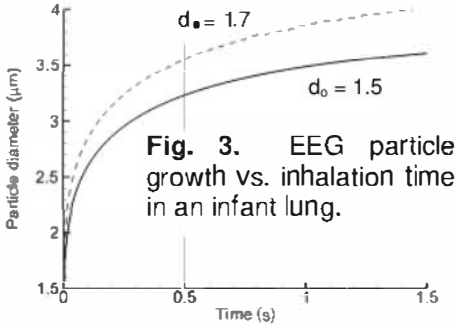

Fig. 3. EEG particle growth vs. inhalation time in an infant lung.

the droplet size achieved can be increased to greater than 3.5 µm by starting with an initial particle size of d_0=1.7 µm and the same formulation, which may also produce sufficiently low upper airway depositional loss.

Surface Tension Characteristics of Surfactant EEG Formulations. The surface tension properties of the Survanta-MN EEG and Curosurf-MN-EEG formulations were characterized using a Bubble Pressure Tensiometer (Kruss) and compared to the marketed Survanta liquid formulation at phospholipid concentrations of 1.5 mg/ml in saline with the addition of 1.5 mM $CaCl_2$. Figure 4 shows a plot of surface tension vs. surface age. A characteristic reduction of surface tension was observed with increased bubble age using this **dynamic measurement technique.** The final surface tension result obtained for Survanta was similar to the static value reported by Bernhard et al. [75]. The Survanta-MN-EEG spray dried powder surface age plot was similar to Survanta liquid indicating that the spray drying process did not appear to affect surface activity of the formulation. The Curosurf-MN-EEG formulation surface age plot indicated a greater surface tension reduction at lower surface ages, despite equivalent phospholipid content. This may reflect the altered surfactant protein composition compared to Survanta and has been previously observed [75]. The measured final surface tension result of 29 mN/m showed good agreement with the value reported by Bernhard et al. [75] at a similar phospholipid concentration. There was no evidence that surfactant protein activity was altered following spray drying or in the EEG formulation.

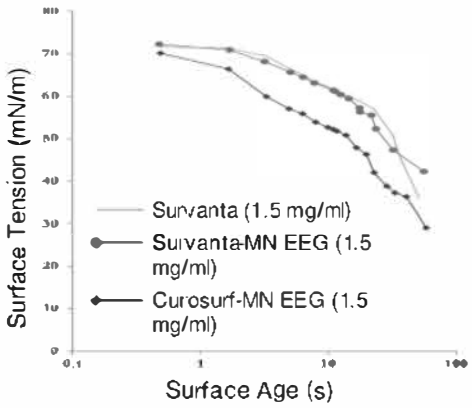

Fig. 4. Surface tension of Survanta, Survanta-MN EEG and Curosurf-MN EEG formulations.

Demonstration of In Vivo Rat Lung Surfactant Depletion Model to Test Efficacy. In order to establish an *in vivo* surfactant depletion (SDp) model, male Sprague Dawley rats (Charles River) were selected according to published methods [76]. Following anesthesia, rats underwent three saline lavage procedures to induce surfactant depletion of increasing severity. Following lavage, lung mechanics measurements were taken with a ScieReq Flexivent FX system. Surfactant depletion significantly reduced compliance as expected (Fig. 5). In addition, control vs. SDp lung resistance increased by a factor of >3x ($p<0.01$) and lung elastance increased by ~3x, at a time point of 10 minutes after final lavage.

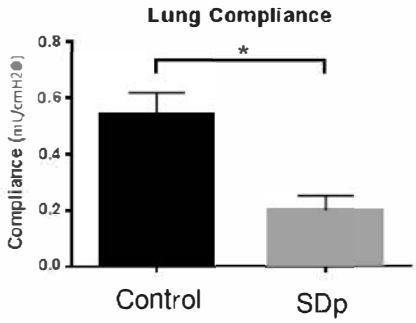

Fig. 5. Lung mechanics data from control and surfactant depleted (SDp) rats. SDp rats had significantly reduced compliance. n=2 per group. *p<0.05

Rationale. Our previous work has introduced and developed the concept of EEG aerosol delivery during oral inhalation [17, 24, 47] as well as with NIV in adults [22, 59, 61]. We have demonstrated that combination particles of water soluble and insoluble drugs can be spray dried, effectively aerosolized, and increase in size over typical inhalation time scales due to hygroscopic excipients and water uptake [16, 18, 19, 24, 47, 63]. However, the generation of EEG formulations with surface active phospholipids and proteins introduces a new level of complexity. The preliminary data indicates that the phospholipids and proteins of Survanta and Curosurf can be successfully spray dried with mannitol and L-leucine excipients. Our studies indicate that L-leucine functions as an effective dispersion enhancer for a variety of therapeutic molecules [19, 24, 47], and that hygroscopic growth can be achieved with a hygroscopic excipient for water soluble and insoluble drugs [17, 18]. Effects of the hygroscopic excipient and L-leucine have not previously been considered on the function of pulmonary surfactant. Theoretically, L-leucine is a weak surfactant [77]. Taeusch et al. [78] reports that hydrophobic molecules smaller than phospholipid surfactant molecules, like L-leucine, **can increase the adsorption of surfactants** and enhance their function. The generated submicrometer-sized combination particle surfactant formulations should provide a MMAD of **approximately 1.5 µm or below** to allow for effective transport through the interface device and infant upper airways. **Size increase due to hygroscopic growth to approximately 3 µm and above** is needed for maximum alveolar deposition [79] in the short ventilation cycle.

To screen the efficacy of EEG surfactant aerosols, a small animal (rat) lung lavage model will be implemented. The purpose is to have a consistent basis for evaluating surfactant efficacy *in vivo* in terms of improvements in lung mechanics compared with instillation. **Due to significant differences in lung anatomies, the rat animal model is not intended to evaluate aerosol delivery efficiency during NIV, which is optimized for an infant in Aim 2 and tested in a correctly sized ferret airway injury model in Aim 3.**

Task 1.1. *Development of quantitative analytical methods to characterize the spray dried surfactant EEG formulations.* Preliminary studies quantitatively characterized the DPPC component of the surfactant formulation. The development of a pharmaceutically acceptable spray dried lung surfactant EEG formulation will require a complete quantitative characterization of the formulation components before and after spray drying, followed by analysis during initial stability testing. The developed LC-MS method (Fig. 2) for the determination of the lipid and protein components of Survanta, Curosurf and Surfaxin will be validated for quantitative analysis. The method appears suitable for bovine and porcine surfactant proteins, however its suitability for synthetic peptide sinapultide remains to be determined and will also be assessed during this task.

Task 1.2. *Use the spray drying technique to produce surfactant EEG formulations containing a hygroscopic excipient (mannitol or sodium chloride) and dispersion enhancer (L-leucine).* Successful preliminary spray drying studies have been performed with animal derived surfactant extracts, Survanta and Curosurf, using conditions optimized for small molecule spray drying. In this task, the spray drying conditions will be optimized for spray drying of surfactant EEG formulations. Three marketed surfactant formulations will be evaluated, animal derived surfactants, Survanta and Curosurf, and a synthetic surfactant, Surfaxin. This task will seek to further develop the spray dried formulation to maximize the surfactant content in the combination particles while maintaining favorable aerosol performance characteristics and hygroscopic growth. A quality-by-design approach will be employed to identify critical formulation parameters by investigating the effects of the % solids concentration, hygroscopic excipient (MN or NaCl) and surfactant:excipient ratio. A similar optimization process will be applied for the critical process parameters such as the spray mesh size, dryer temperature and gas flow rate. Phospholipid and surfactant protein content, primary particle size, surface tension reduction and aerosol performance of the spray dried combination particles will be used as critical quality attributes for optimization and will be assessed using LC-MS analysis (Task 1.1), *in vitro* aerosolization (Task 1.3), bubble pressure tensiometry (Task 1.4) and SEM (Task 1.4). This task will deliver an understanding of the spray drying experimental design space that will enable production of Surfactant-EEG combination particle formulations with varying drug:excipient composition that will be characterized in subsequent *in vitro* studies.

Task 1.3. *Test the aerosolization and growth characteristics of surfactant EEG formulations using a low flow aerosol generator and humidity exposure system.* The aerosol performance and hygroscopic growth characteristics of the spray dried EEG formulations will be assessed using previously developed methods [18]. Formulations will be loaded into the 2.0-232 inline DPI low flow device and actuated at a flow of 5 LPM (L/min) into the NGI operated with dilution air at 15 LPM to determine the aerosol performance [22]. The emitted dose from the inline DPI, the MMAD, geometric standard deviation (GSD) and FPF will be evaluated for the study formulations using DPPC as the quantitative marker. Optimization will include investigation of the effects of the powder formulation loaded dose in the DPI capsule (20, 30 and 40 mg) on the delivery time. This task will identify EEG formulations with critical performance attributes suitable for development as a high efficiency dry powder surfactant formulation for use in infants. The powders should generate an emitted dose of >80% of the nominal dose loaded into the capsule and a $FPF_{<5\mu m}$ >85%. Hygroscopic growth will be measured by drawing the aerosols generated at 5 L/min from the inline DPI into a hygroscopic growth tube with wetted walls designed to simulate 0.5 s lung exposure [16, 18]. The tube will be held at 37°C and 99% RH to simulate expected lung conditions. Following passage through the tubular geometry, the aerosols will be delivered to the NGI operated with equilibrated dilution air at 15 LPM for particle sizing. The tubular geometry and the NGI will be placed in an environmental chamber to maintain constant temperature and RH conditions. The initial and final particle sizes will be used to calculate growth ratios as a measure of hygroscopic size increase.

Task 1.4. *Test the in vitro functional and physico-chemical characteristics of surfactant EEG formulations.* The bubble tensiometer method will be used to characterize the dynamic surface tension of the spray-dried formulations produced in Task 1.2 together with the adsorption and diffusion coefficients. Experiments will be performed at 37°C and will employ controls of the marketed Survanta, Curosurf and Surfaxin formulations, together with solutions of the hygroscopic and dispersion excipients. Lung-like surface expansions will be evaluated using the captive bubble tensiometer method in which, in addition to surface tension, surface compressibility will be reported for the test formulations. The formulations will also be characterized by: (i) the particle size using SEM, Sympatec HELOS RODOS and Malvern Mastersizer, (ii) the particle crystallinity using Differential Scanning Calorimetry (DSC), (iii) the water content of the combination particles using Karl Fischer (KF) Titration and Thermogravimetric Analysis (TGA), (iv) the true density using helium pychnometry. Following the analysis in Tasks 1.2 - 1.4, 3 leading surfactant formulations will be selected based on optimal surfactant activity, aerosolization, and high growth potential. These three lead formulations may vary based on the liquid surfactant base or formulation.

Task 1.5. *Assess in vivo efficacy of the spray dried EEG surfactant formulations delivered as an aerosol compared with liquid bolus instillation in a rat surfactant depletion model based on blood gas levels and respiratory mechanics and select a lead formulation.* In order to confirm the efficacy of the EEG surfactant formulations delivered as an aerosol following preparation as powders by spray drying, we will use a rat surfactant depletion model as described previously [76, 80]. The purpose of these experiments will be three-fold: (i) confirm the *in vitro* observations of surfactant functionality following spray drying of the surfactant EEG formulations using an *in vivo* model (ii) select a leading formulation based on minimum effective dose in an animal model and (iii) compare dosimetry of aerosol delivery with instillation. As with our preliminary data, rats will be lung lavaged 3 times with warmed saline (10 mL/kg) to deplete lung surfactant. Following lavage, rats will be dosed with EEG surfactant aerosol formulations (3 dose groups) delivered as an aerosol or delivered as an instilled bolus. The aerosol will be generated and administered using a microsprayer and metal endotracheal attachment with a small volume air pump to avoid deposition in the highly complex rodent nasal airway. Control animals will be treated with the marketed liquid surfactant formulation as a liquid bolus instillation using standard procedure for infants, which involves intubation and liquid bolus delivery. After surfactant administration, rats will be chemically paralyzed and mechanically ventilated with a Flexivent FX equipped with a rat module (SciReq). Over a period of 4 hours, survival and standard lung mechanics will be measured including resistance, compliance, and PV loops. Pulse oximetry and heart rate will be measured during anesthesia. Blood gas measurements will be obtained before injury, after surfactant depletion, and after treatment and mechanical ventilation using an arterial catheter and a Siemans blood gas analyzer.

For controls, instilled liquid surfactant will be administered at the clinically recommended dose, e.g., 4 ml/kg for Survanta which is equivalent to 100 mg/kg of phospholipids. The EEG aerosol surfactant dose (in terms of phospholipid content) will initially be delivered at 1/10 the instilled dose for each EEG formulation to establish efficacy of the aerosol administration (group 1). The minimum effective dose will be determined using lung compliance as the primary metric. If lung compliance is not recovered to within 15% of the initial value prior to surfactant depletion, the aerosol dose will be increased to 25% of the initial dose. Similarly, if lung compliance is within 15% of the initial value, the dose will be decreased to 25% of the initial dose (group 2). This process will be repeated to determine the third dosing level (group 3), with increased doses level of 25% of the previous dose or decreased dose level of 25% of the previous dose. In our experience, lung compliance is a more sensitive measure to pulmonary function and will decline significantly earlier than changes in blood gasses can be detected. Selection of the leading aerosol surfactant formulation will be based on the formulation with the lowest effective aerosol dose as assessed using blood gas and lung mechanics metrics. A sample size of 10 male and female animals will be treated in each EEG surfactant aerosol formulation group with corresponding control liquid bolus groups for each of the commercial surfactants tested (e.g., Survanta, Curosurf or Surfaxin). This sample size was chosen based upon preliminary data for a t-test (assuming normality) with a significance level $\alpha = 0.05$ and a desired power of 80%. Statistical differences between test formulations and controls will be assessed at a level of $P<0.05$ using a t-test, while the effects of dose and EEG formulation will be compared with one or two way ANOVA with pair-wise comparisons as appropriate.

Potential Problems and Solutions. Initial studies indicate no stability issues with the Survanta and Curosurf EEG formulations; however, a list of alternate excipients may be considered if needed. Stability will be addressed by selection of appropriate temperature conditions together with packing material to minimize the effects of environmental exposure during storage. Stability studies will be initiated under ambient and accelerated conditions to determine chemical and functional performance over time when stored in capsule-based primary packaging. Preliminary studies have shown that refrigerated powders are functionally active after 2 weeks storage following spray drying, which was the longest time period tested in preliminary studies. Should any of the products become unavailable, alternates (e.g. lyophilized Surfaxin) may be utilized.

C.2 Specific Aim 2: Develop and optimize a device for generating and administering surfactant aerosols to infants using the noninvasive nose-to-lung (N2L) route and achieving high efficiency lung delivery.

Preliminary Data: *Performance of 3D Rod Array Inline DPI Devices Across a Range of Flows.* The 3D rod array inline DPIs developed by Behara et al. [22] consisted of a flow control orifice, capsule chamber, and 3D rod array as shown in Figure 6. The devices are actuated either with compressed air or a manual ventilation bag. Behara et al. [22] reported that the best performing device for use with high flow nasal cannula (HFNC) therapy in adults had a flow control orifice diameter of 2.3 mm with a 3-4-3 rod array pattern (2.3-343 device). Formulations of spray dried albuterol sulfate (AS) or ciprofloxacin (Cipro) with mannitol (hygroscopic excipient) and L-leucine (dispersion enhancer) were generated [19] and considered in the three devices shown in Table 1. The devices were actuated by a manual ventilation bag and the size of the flow control orifice determined

the measured delivery flow. With the use of the flow control orifice, the flow rate was not sensitive to bag pressure. Emitted doses from the 3D rod array DPIs were all ~70% or higher. The 2.0-232 device was observed to produce a high quality aerosol ($FPF_{<5\mu m}$ >80%) with a flow of only 5.0 LPM. Further optimization of this device is expected to result in reductions of MMAD at even lower flows.

Evaluation of EEG Delivery with a 4 mm Endotracheal Tube (ETT) in an Infant Model. CFD simulations were employed to evaluate the effectiveness of EEG aerosol delivery to an infant (6 months and 8 kg) receiving invasive mechanical ventilation. Ventilator settings were based on Walsh et al. [74] and included 40 br/min, a tidal volume of 56 ml, and a tracheal flow of 6.7 LPM. The airway geometry of a 6 month old infant based on the study of Phalen et al. [81] was implemented in the stochastic individual path (SIP) approach [82-84] to simulate deposition throughout the tracheobronchial (TB) airways. For EEG delivery, a dry powder aerosol was delivered through a port in the streamlined wye. The EEG aerosol had an initial MMAD of 1.8 µm and was composed of particles containing 25% AS (model drug) and 75% NaCl (hygroscopic excipient) by mass. Warm and humid air (37 °C and 100% RH) was supplied to the inspiratory ventilator line at a flow rate of 6.7 LPM and the walls of the infant airways were assumed to be at 37 °C and 100% RH. CFD predictions of deposition and aerosol size growth were previously validated with *in vitro* data [16, 17, 56, 64, 83]. CFD predictions of depositional losses in the ventilation unit including the ETT were acceptably low (~10% total). Significant size increase of the initial 1.8 µm aerosol was observed throughout the airway model. Droplets of approximately 5.0 µm were predicted to exit B15 and enter the alveolar space. Drug deposition fractions (DF) in the ventilation unit (loss), TB, and alveolar airways were estimated to be 8.8, 48.3, and 43.9%, respectively.

Validation of Complete-airway CFD Simulations in Adults. Our group has recently developed complete airway CFD simulations of pharmaceutical aerosols in adults using a stochastic individual pathway approach [82-84]. These studies are the first to use CFD modeling to simulate the transport of pharmaceutical aerosols from the site of origin inside the inhaler to deposition throughout the lungs. Important features captured by the model include the jet effect from the inhaler, transient inspiration, heterogeneous lung lobe ventilation, hygroscopic aerosol size increase, and accurate alveolar wall structure and wall motion. Comparisons of model predictions with 2D *in vivo* gamma scintigraphy imaging [85, 86] resulted in <15% relative error across the entire airways divided into three regions defined by the limitations of the experimental measurements. These comparisons were established for multiple inhalers (Respimat and DPI) [85] and at multiple DPI inhalation flow rates [86].

Rationale. Improved delivery efficiency of lung surfactants in this project is based on (i) submicrometer EEG powder formulations to effectively navigate the delivery system and upper airways, (ii) streamlined patient interfaces, where both techniques were developed by our lab [18, 26, 55, 58, 60], together with a new positive pressure N2L aerosol delivery system. We have previous developed 3D rod array-based inline DPIs [24, 47, 63] and proven this technique to be highly effective for deaggregating submicrometer EEG powders [23]. These devices improve performance compared with marketed products in terms of reductions to MMAD and mouth-throat (MT) deposition, increases in FPF, and insensitivity to flow rate [24, 47, 63]. The combination of streamlined ventilation components and a 3D rod array inline device with an EEG powder formulation results in a nasal cannula emitted dose of >75% for high flow cannulas in adults and <5% nose-mouth-throat (NMT) drug deposition [59, 61]. The low flow DPI preliminary data demonstrates effective deaggregation of powders at lower flow rates by modifying the dimensions of the DPI device. In this aim, the 3D rod array inline devices (and an alternative capillary device) are further optimized for use with surfactant EEG formulations at even lower flow rates. Streamlining concepts are applied to improve aerosol transport for nasal interfaces to enable N2L delivery. During realistic cyclic ventilation of a **newborn infant, lung delivery efficiencies of 70%** are targeted with 10 mg of phospholipids delivered in under 30 seconds. For **preterm infants** of 1600 g (31 weeks gestational age), lung delivery efficiency of **60%** will be a targeted goal.

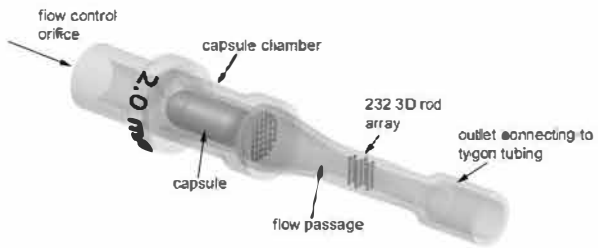

Fig. 6. 3D rod array inline DPI with a 2.0 mm flow control orifice and 232 rod array pattern.

Table 1. *Performance of 3D rod array devices [mean (SD)].*

Device	*2.3-343*	*2.3-212*	*2.0-232*
Drug	*AS*	*AS*	*Cipro*
Flow Rate (LPM)	*14.7 (0.9)*	*11.6 (0.6)*	*5.0 (0.0)*
ED (%)	*75.3 (2.5)*	*68.8 (4.4)*	*76.6 (3.9)*
$FPF_{<5\mu m:ED}$ (%)	*65.4 (6.4)*	*60.2 (8.5)*	*85.3 (3.1)*
MMAD (µm)	*1.6 (0.07)*	*1.6 (0.07)*	*2.2 (0.02)*

Task 2.1. *Develop and characterize a series of inline DPIs based on 3D rod array technology that can effectively generate aerosols at an optimal flow rate of 2-4 LPM using a CFD and in vitro approach.* Design of the devices will consist of CFD simulations, rapid prototyping, and *in vitro* testing. CFD simulations of the devices will implement best practices and methods previously described in our publications on device design [23, 26, 64, 68, 87]. For the DPIs, transient and compressible flow simulations will be conducted with the Low Reynolds Number k-ω turbulence model [88]. Using this approach, we found a strong quantitative correlation between the newly developed non-dimensional specific dissipation (NDSD) design parameter and aerosol deaggregation compared with *in vitro* experiments [23]. Preliminary experiments indicate that modified designs of the 3D rod array inline DPI (Fig. 6) with a flow control orifice of 1.0 mm produces rapid fluctuations at 2 LPM. The optimal 3D rod array inline device will be produced using an in-house rapid prototyper (3D printer; Viper SLA, 3D Systems) using high quality resin (Accura 60) [24, 63].

As an alternative to the vibrating capsule 3D rod array design (Fig. 6), a 2nd inline DPI will be developed that passes all of the air used to form the aerosol through the capsule. As shown in Fig. 7, inlet and outlet capillaries will pierce into the capsule to form a continuous flow passage. Jetting air motion through the capsule will be used to deaggregate and emit the powder from the device. CFD simulations together with *in vitro* experiments will be used to optimize the design [23]. This low air volume (LV) DPI will be actuated with 10 ml or larger air boluses delivered from a positive pressure supply (syringe bank described below).

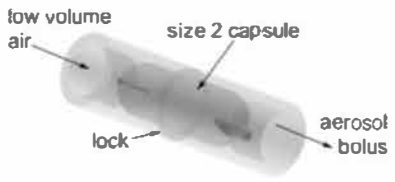

Fig. 7. Inline LV-DPI

Table 2. Typical ventilation parameters [74].

	Preterm (1600 g)	**Full term (3550 g)**
Period (s/br)	1.5	2.0
T_{inhale} *(s)*	0.4	0.5
V_{tidal} *(ml)*	9.6	28.4
$Q_{trachea}$ *(LPM)*	1.4	3.4

In vitro experiments for both DPI designs will be used to evaluate emitted dose, device retention, and aerosol size exiting the DPI based on cascade impaction with the NGI as described in previous work [18, 22]. Rapid screening of device modifications will be possible using the INHALER accessory for the Sympatec laser diffraction sizer. Surfactant EEG formulations (selected in Aim 1) will be loaded into the capsules, to achieve delivery of 10 mg phospholipids to the lungs. **Animal models report that aerosolized surfactants are effective at doses much lower than with liquid instillation [6, 89], as with Lewis et al. [90] who demonstrated respiratory conditions improved after aerosolization of 6 mg/kg in a lamb model.** Higher doses can be achieved by administration of additional capsules. Characteristic inhalation conditions for a preterm (1600 g) and full term infant are displayed in Table 2. As presented in Task 2.2, a positive pressure sealed system will be used to deliver the aerosol and control infant respiration allowing for deep inhalation breaths during dose administration. For the 3D rod array system, preterm flow parameters will be Q=2 LPM; V_{inhale} = 10-20 ml; T_{inhale} = 0.3-0.6 s; and for the full term infant Q=4 LPM; V_{inhale} = 30-60 ml; T_{inhale} = 0.5-0.9 s. For the capillary device, where delivered air volume is the primary metric, flow parameters are preterm infant: V_{inhale} = 10-20 ml; T_{inhale} = 0.3 s; Q = 2-4 LPM; and full term infant: V_{inhale} = 30-60 ml; T_{inhale} = 0.6 s; Q = 3-6 LPM. The delivery device (Task 2.2) will incorporate a pressure relief safety value to ensure that a maximum pressure is not exceeded. Targeted device performance is an ED of >70% with $FPF_{<5\mu m}$ >80%, MMAD <1.5 μm and 90% emptying of the capsule in 30 seconds or less. Once this targeted performance is achieved, devices with larger capsules will be tested up to 90 mg total mass. A comparison of the devices will be conducted with a **leading device** selected for further testing, based on highest emitted dose and lowest MMAD.

Task 2.2. *Develop a N2L delivery platform capable of high efficiency lung delivery and controlled infant respiration.* To address the problems associated with aerosol delivery without intubation and avoid challenges associated with trying to interface with the wide range of existing NIV systems, we propose a **new stand-alone N2L aerosol delivery device** for infants that can rapidly and efficiently deliver high doses of aerosol to the lungs and control respiration to achieve peripheral lung deposition. The device consists of a streamlined nasal cannula interface that forms a seal with the patient's nostrils, an inline DPI unit, and a positive pressure air source, as shown in Fig. 8 and described below. The device will be operated by a respiratory therapist (RT; as with current surfactant instillation) and can administer rescue or prophylactic doses of aerosolized surfactant.

The patient interface will be short bilateral nasal prongs that are streamlined to minimize aerosol depositional loss. Multiple prong sizes will be developed to accommodate infants ranging in weight from 1250 g to > 3000 g. The inner and outer diameters of these prongs will be based on the well accepted Hudson RCI nCPAP interface, which provides dimensions for 4 different prong sets in the desired weight range [91]. The smallest prongs will have an internal diameter of 3.0 mm, which is sufficiently large for the passage of EEG

aerosol at the desired flow rates. To ensure an airtight seal, small foam cones will be placed on the prongs. Distance between the prongs will also be based on the Hudson RCI product, but may make sealing difficult. To address this issue, we will develop a flexible joint between the prongs that can accommodate a 50% expansion of the inter-prong spacing. Fit of the 4 prong sizes will be tested across *in vitro* nasal models of 5 infants in each of the following weight ranges: 1600 g, 2600 g, and 3350 g. The models will be developed from pre-existing scans at Children's Hospital of Richmond. The design of the streamlined cannula shape (similar to Fig. 9) will be optimized using CFD, as in our previous work. The streamlined cannula shown in Fig. 9 (side inlet version) has an inner diameter of 4 mm and in-house *in vitro* experiments indicated a delivery efficiency of 99.2% for an EEG aerosol at 5 LPM airflow.

The delivery device body will consist of the nasal cannula connected (by Luer lock) directly to the inline DPI. Either inline DPI may be used, but the capillary based design (Fig. 8) provides the advantage of automatically piercing the capsule upon loading. A pressure relief (pop-up) valve will be integrated to avoid over pressurizing the lungs. An exhalation port will also be included, which is covered by the RT's thumb during delivery and uncovered for exhalation.

A critical element of the device is the use of a sealed system and positive pressure gas. One option for controlled small gas volumes is a simple bank of syringes (Fig. 8). Each syringe can be emptied in 0.3 to 0.6 s, which is consistent with typical neonatal inhalation. The syringe provides a controlled amount of inspiration, which can safely be up to 2x the typical tidal volume for the ≤5 breaths needed for aerosol delivery providing the required deep inspiration. The pressure relief valve ensures that the lungs are not over pressurized. The positive pressure system will also allow for a brief breath hold period controlled by the RT.

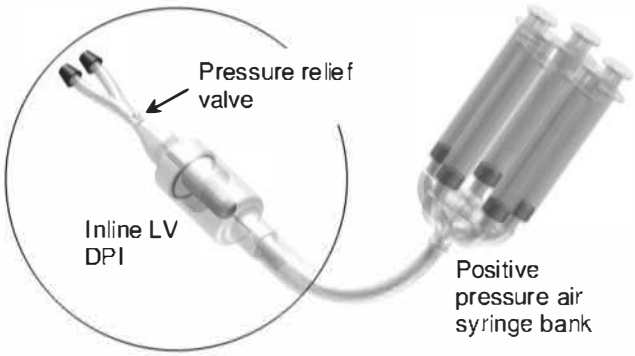

Fig. 8. New positive pressure N2L aerosol delivery system for an infant.

To operate the system, the RT will measure (with a template) and attach the correctly sized nasal cannula to the device. They will then insert the capsule, close the device, and insert the prongs a short distance into the nostril to form a seal. The DPI body is held in one hand and the air syringe bank in the other. After an exhalation, the RT covers the exhalation port and quickly actuates one syringe (0.3 - 0.6 s), enforces a breath hold for ~1s, and then releases the exhalation port (~3 s). The RT will also need to ensure that the infants mouth stays closed. Up to 5 cycles are required to empty the capsule taking a total of approximately 25 s. The preferred form of NIV is then connected to the patient.

Fig. 9. NMT infant upper airway model.

Task 2.3. *Evaluate performance of the N2L delivery platform based on lung delivered dose (tracheal filter) using an in vitro model and realistic ventilation conditions.* Realistic infant nose-mouth-throat (NMT) models will be developed based on pre-existing scans of preterm (~1600 g) and full-term (~3550 g) infants available at Children's Hospital of Richmond. These models will be constructed using in-house rapid prototyping as with models previously developed by our group [59, 61] and will continue to the trachea. As shown in Fig. 9, airway surfaces will be extracted from the CT scans using the segmentation and 3D model rendering software Mimics 18 (Materialize). The 3D airway surface geometries will be converted to hollow models of the airways and produced using in-house rapid prototyping (3D printing) capabilities on a Viper SLA machine in Accura 60 resin (3D Systems). At the trachea, the models will be connected to a pediatric test lung, that will create inspiratory and expiratory breaths consistent with values in Table 3. A filter will be placed at the tracheal outlet prior to the test lung to capture the lung delivered dose. Surfactant aerosol deposition (using DPPC as a quantitative marker) in the models and on the filter at the exit of the trachea will be quantified using the previously developed method (Task 1.1). Aerosol delivery using the N2L delivery platform (Task 2.4) will be evaluated at various phases of the respiratory cycle to determine the efficiency of the controlled respiration delivery method. Depositional losses of <20% and <10% in the preterm and full-term NMT models will be considered targets for successful aerosol lung delivery leading to the previously stated lung delivery efficiency goals of 60% and 70%.

Task 2.4. *Develop a CFD model of aerosolized surfactant lung delivery (to the trachea) under realistic ventilation conditions and use the model to optimize performance of the N2L systems.* A CFD model of infant airway geometries (preterm and full term) extending to the trachea (similar to Fig. 9) will be developed. The model will include simulations of aerosol transport, hygroscopic growth, and deposition as performed in our

previous studies [21, 56, 59, 62]. It is noted that this modeling approach has previously agreed well with *in vitro* predictions of aerosol growth and deposition for condensational growth delivery [21, 56, 59, 62]. Comparisons with the *in vitro* results from Task 2.4 will help to further confirm the predictions. Model results will in turn be used to better understand NMT depositional surfactant losses and respiratory losses.

Task 2.5. *Develop a new CFD model of the complete infant airways, validate with existing in vivo data, and apply the model to optimize EEG surfactant aerosol delivery.* Our group has recently developed complete-airway (Fig. 12) CFD models of adults [82-84] and infants [92] and validated regional medical aerosol deposition in the adult model with existing *in vivo* data for multiple inhalers [85, 86]. In this task, the infant model will be extended and applied to preterm and full term neonates. As with Task 3.3, pre-existing CT scans will be used to create the airway geometries through bifurcation B3. The stochastic individual pathway technique, developed by our group [82-84], will then be used to evaluate transport and deposition in the TB airways. Models of infant acinar airways will be developed that include wall motion [93]. As developed in our previous studies, the complete airway simulations will account for polydisperse aerosol size, cyclic breathing, turbulent dispersion, and hygroscopic growth, as appropriate in the different airway regions [21, 62]. The new complete airway models will be validated with the existing *in vivo* aerosol deposition studies of Fok et al. [43] and Amirav et al. [94]. Upon successful validation, the new complete-airway models will be used to concurrently assess surfactant aerosol delivery and optimize deposition in the alveolar region based on suggested modifications to initial particle size, hygroscopic content, and gas delivery timing.

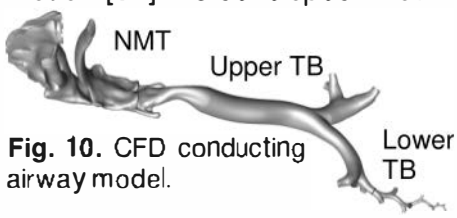

Fig. 10. CFD conducting airway model.

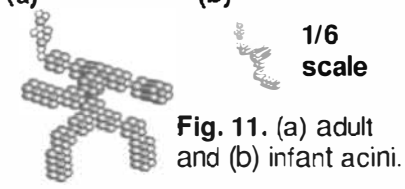

Fig. 11. (a) adult and (b) infant acini.

A significant challenge in developing the complete airway models of preterm and term infants will be extending the airway models beyond B3. Considering the TB region, the number of conducting airway generations is set in this age range. The airway growth correlations of Phalen et al. [81] include a 3200 g infant and can therefore be used for the 3550 g TB case. These correlations are highly linear with infant height (R^2 = 0.99) and it will therefore be assumed that they can be extended to the preterm infant. Regarding the infant alveolar region, significant changes rapidly occur with age in the areas of alveolar (i) scale, (ii) shape, and (iii) number/occurrence. We will use the alveolar data of Hislop et al. [95] and the volume filling [93, 96] method to approximate these effects for the two infant categories considered, as with [96]. Considering scale, Fig. 11 illustrates the size difference between an adult acinar unit and that of a 3550 g infant, and missing alveoli.

Potential Problems and Solutions. We have also previously generated EEG aerosols using soft mist inhalers [17] and pioneered the capillary aerosol generation (CAG) device [97-99], which can produce dry particle EEG aerosols [18, 99], providing additional design alternatives for small EEG particles if needed.

C.3 Specific Aim 3: Adapt the N2L aerosol device and test EEG surfactant aerosol efficacy in an infant-size ferret model compared with surfactant instillation in terms of oxygenation, lung distribution and histology.

Preliminary Data. *Delivery of Therapeutic Aerosol to a Ventilated Ferret Model.* In a previous study, Rubin et al. [100] implemented a spontaneously breathing ferret model of airway inflammation and therapeutic aerosol delivery. Inflammation was introduced with lipopolysaccharide and the anti-inflammatory characteristics of oral and aerosol dapsone were evaluated. Ferrets weighed 1.3-2.0 kg (comparable to a 31 week gestation infant) and were intubated with an uncuffed 3 mm ETT. Dapsone was delivered as an aerosol using a commercial jet nebulizer, which delivered low doses (estimated to be 0.1-1.0 µg) due to poor delivery efficiency (~1% of the nebulized dose). Outcomes assessed included neutrophil accumulation, mucus secretion, and mucus clearance. This study illustrates our ability to use a ferret model with comparable size to a preterm infant, deliver an aerosol with a commercial nebulization system, and evaluate lung function and histology.

Rationale. In Task 1.5 we establish the *in vivo* efficacy of different EEG surfactant formulations and select a leading formulation. However, lung delivery efficiency of the aerosol is not considered. Aim 2 develops an effective delivery strategy and device for N2L administration of the aerosol. In this aim, we test both the lung delivery efficiency and therapeutic efficacy in an appropriately sized animal model with lung injury. Based on the study of Phalen et al. [101] for multiple animal species compared with humans, ferrets had the most similar tracheal structure, bifurcation shape, length/diameter ratio and number of respiratory bronchioles. Several studies have also made morphometric measurement of ferret airways [102, 103]. Based on airway size measurements, the first three airway diameters of an infant weighing 1600 g [81] are 4.9, 3.8 and 2.9 mm, and are closely matched with a 56 day old ferret with values [102] of 5.1, 3.8 and 2.8 mm. Considering our previous 25 years of experience with ferret models, we selected ferrets for *in vivo* aerosol delivery and efficacy testing.

Task 3.1. *Adapt the aerosol generation device for high efficiency lung delivery to a ferret animal model.* The aerosol delivery device will first be adapted for N2L delivery to a ferret. The primary change will be developing a nasal cannula interface for the curved slit nostril inlet. A ferret nasal model will be developed from CT scans of a ferret cadaver in a small animal CT. As with the infant nasal models, the scan will be converted to a 3D model and prototyped with a 3D printer in a flexible material. Systems will be developed for a young adult ferret weighing approximately 1600 g (range of 1000 to 2000 g). Appropriate gas delivery volumes equal to 2x the standard tidal volume will be implemented. Aerosolization performance and cannula emitted dose of this model system will be tested with *in vitro* experiments as in Task 2.1.

Task 3.2. *Evaluate therapeutic efficacy (oxygenation) of the optimized EEG aerosol formulation and delivery device based on N2L administration to an infant-size spontaneously breathing ferret model with surfactant depletion.* Spontaneously breathing ferrets in a weight range of approximately 1000 to 2000 g will be evaluated for N2L surfactant administration. Considering that the nasal structure of the ferret is considerably more complex than that of an infant, N2L delivery in a ferret provides a significant challenge and should provide a conservative estimate of lung delivery efficiency in an infant with similar lung size. Surfactant depletion will be achieved using lung lavage with warmed saline as performed in Task 1.5 and repeated until peripheral capillary oxygen saturation (SpO_2; measured with foot oximetry) reaches 0.90. We view spontaneous respiration to be more similar to NIV than a mechanically ventilated ferret model. As a result, it is not possible to determine airway compliance in these experiments. However, animal model studies [15] have observed significant increases in oxygenation with aerosolized surfactants.

Following lung lavage, ferrets will receive either the lead EEG aerosol formulation delivered using the optimized device and strategy developed in Aim 2 and modified for ferrets in Task 3.1 or the corresponding commercial surfactant (e.g. Survanta) instilled as a liquid bolus using the manufacturer recommended procedures (control). Over a period of up to 1 hour, foot oximetry will be recorded to measure SpO_2 as the primary metric, together with respiration rate. The primary outcome variable will be the time to normalization (SpO_2>0.95) of oxygen saturation following surfactant administration. Instilled liquid surfactant will be administered at the clinically recommended dose (typically 100 mg/kg of phospholipids). The lead EEG aerosol formulation will be administered at the minimum effective dose (mg/kg phospholipid) determined in Task 1.5, corrected for the expected losses in the upper airways of the ferret, determined using the *in vitro* model from Task 3.1. A statistical comparison of the SpO_2 levels as a function of time post administration will be made using repeated measures ANOVA with significance assessed at a level of $p<0.05$. A sample size of 10 male and female animals will be treated in the aerosol formulation group with a corresponding control liquid bolus group. This sample size was chosen based on the "resource equation" method of estimating sample size.

Task 3.3. *For a best case aerosol surfactant dose and standard of care liquid instillation, compare lung histology and lobar distribution of phospholipids.* Previous aerosol delivery studies have demonstrated significant reductions in lung injury, improved alveolar opening, and more homogeneous lung distribution of phospholipids with aerosol surfactant delivery compared with instillation [15, 52, 53]. Similar confirmation is required for the new EEG aerosol surfactant product, which is expected to improve upon previous aerosol surfactant results. For the control (instillation) and best aerosol delivery case established in Task 3.2, lungs of n=3 animals in each group will be removed and fixed. The lung histology methods for ferrets implemented in the Rubin lab [100, 104] will be adapted for evaluating metrics relevant to surfactant delivery. Histopathology will include: alveolar inflammation based on lung injury score [52], alveolar edema and hemorrhage [52], alveolar expansion index [53], and number of open exchange units [53]. In addition, inter-lobar distribution within the lungs of instilled dose compared with aerosol delivery will be determined for n=3 animals in each group. The six lung lobes of each animal will be removed and evaluated for surfactant content using the HPLC methods established in Aim 1. Statistical differences will be assessed as in Task 3.2.

Potential Problems and Solutions. Ferrets represent a new, and we expect robust [100, 104], appropriately sized animal model to evaluate surfactant delivery and efficacy during spontaneous respiration in an infant. However, difficulty may arise with balancing sufficient lung injury for evaluating efficacy with survival. If ferret survival is a problem, we will mechanically ventilate the ferrets similar to Task 1.5. If we are unable to induce sufficient lung injury with airway lavage, we will consider other methods such as lipopolysaccharide [100], hydrochloric acid [53], or meconium instillation.

C.4 Timeline. It is estimated that each SA will require approximately 16 months to perform. For each SA, planning, testing, execution, and analysis phases are estimated to require 1, 2, 12, and 1 months, respectively. As a result, a total project period of 4 years is requested.

Protection of Human Subjects

Pre-existing CT and MRI infant nasal and chest scans will be reviewed to construct the upper airway models required in the project. High quality scans of target age ranges including full term and pre-term infants will be identified and selected. Some identifying information may be present on the scans when viewed, making this Human Subjects research. All identifiable information will be removed from each scan selected. For each selected scan, the only information recorded will be subject sex, gestational age in 1 week increments, age in 1 week increments, race, height in 1 cm increments and weight in 0.1 kg (100 g) increments, if available. A code will not be created that can be used to identify the subjects. The selected scans will be analyzed to compute airway dimensions. Scans representing average or a range of airway dimensions will be used to construct three-dimensional surface models of the respiratory tract. All selected and de-identified scan information will be stored on a secure password protected computer that only the investigators can access.

This Human Subjects research falls under Exemption 4. Under HHS regulations at 45 CFT 46.101(b), Exemption 4 states:

"Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects."

This exemption applies because:

1. Only pre-existing scans will be accessed.

and

2. The investigator will only record the following information for each saved scan: subject sex, gestational age in 1 week increments (e.g., 1 week, 2 weeks, etc), age in 1 week increments, race, height in 1 cm increments, and weight in 0.1 kg increments, if available. A code will not be created that can be used to identify the subjects. All selected and de-identified scan information will be stored on a secure password protected computer that only the investigators can access.

Vertebrate Animals

Rats

1. For whole animal efficacy experiments, we will use 180-250g male and female Sprague-Dawley rats. An equal number of male and female rats will be utilized in each treatment group. We anticipate needing 10 animals per group based upon power analysis with a power of 0.8 from our preliminary data. Animals will be randomly assigned to treatment groups. Rats will be surfactant depleted. Each group will be treated with aerosolized surfactant or a liquid bolus. For each of the 3 drugs, there will be 3 study groups plus a liquid bolus control. This will result in 120 rats total. The dosing schematic is shown in the table below:

		Study 1	**Study 2**	**Study 3**
	Control	Aerosol (1/10 dose)	If < 15% of initial value then increase dose by 25 %	If < 15% of initial value then increase dose by 25%
			If within 15% of initial value then decrease dose by 25 %	If within 15% of initial value then decrease dose by 25 %
Survanta example	100mg/kg	10mg/kg	12.5 mg/kg or 7.5 mg/kg	15.625 mg/kg or 5.625 mg/kg
	# of animals	# of animals	# of animals	# of animals
Survanta	10	10	10	10
Curosurf	10	10	10	10
Surfaxin	10	10	10	10

2. Rats are the clear choice for the whole animal experiments for the proposed study based upon published studies of aerosol drug deposition. While rats cannot provide evidence of safety for human trials, they will provide preliminary results as to the immunogenicity and deposition of the drugs. There is no phylogenically lower species available that has lung architecture and immune system necessary to test our parameters.

3. Minimization of pain and distress. Rats will be monitored daily for any signs of pain or distress. If pain or distress is noted, rats will be euthanized. Standard veterinary care will be performed at Virginia Commonwealth University prior to experimentation. Rats that are given treatments will be monitored closely for signs of discomfort. If signs of abnormal discomfort are observed, rats will be euthanized. Animals will be housed in the animal facility at the Virginia Commonwealth University barrier facility vivarium (Richmond, VA). Animals are kept in cages and maintained in a temperature and air exchange controlled laboratory environment with 12 hour light-dark cycles for at least 7 days before any experiments begin. The experiments are conducted under IACUC and NIH guidelines and are approved by the animal care committee before the experiments commence. Animals are monitored daily and any animals that appear to be moribund are examined for activity level and body temperature using a digital, non-contract infrared thermometer. Disposal of animal remains, pathological specimens, and animal bedding will be handled as required by the State mandated biomedical waste tracking act.

4. The rats will be euthanized with sodium pentobarbital overdose followed by exsanguination.

Ferrets

1. For surfactant aerosol studies, we will use 1-2 kg adult ferrets of either sex (retired breeders). As this is a pilot study, we plan to evaluate 10 animals per group (based on the "resource equation" method of estimating sample size), randomized to receive either commercial surfactant (Survanta) by endotracheal tube instillation or EEG surfactant by nasal aerosol inhalation. The instilled dose will be derived from the labelled prescribing instructions based on body weight. The aerosol dose employed will be derived from the initial proof of concept rat studies and will be scaled by body weight. The primary outcome variable will be the time to normalization ($SpO2 > 0.95$) of oxygen saturation following surfactant administration. The experiment will be terminated in 1 hour if oxygen saturation does not return to normal, and the minimum and maximum oxygen saturation will be recorded.

   Ferrets will be anaesthetized with xylazine and ketamine and kept in a state of anaesthesia light enough to maintain oxygen saturation, measured continuously by foot oximetry, while breathing spontaneously through a 3.0 endotracheal tube placed at mid trachea. The lungs will be depleted of surfactant by repeated washing with isotonic saline lavage until SpO2 is between 0.90 and 0.92

2. The ferret is a robust and well established animal model for studying airway disease. Not only are ferret airways physiologically and anatomically similar to infant airways, but the ferret is a similar size to a premature newborn infant with an airway the size of a term infant. The Rubin lab has 20 years of experience using this animal for a wide variety of studies related to aerosol deposition and airway disease and we are considered a reference lab for similar studies. We have developed validated instruments to evaluate airway injury and repair in the ferret that will be used to assess histologic changes in these animals following lavage and surfactant depletion.

3. Minimization of pain and distress. Ferrets will be monitored during the study for signs of pain or distress. It is anticipated that the mild hypoxaemia induced by whole lung lavage will cause tachypnoea but not pain. All procedures will be performed under sedation and animals will be euthanized at the end of each investigation.

   AALAC approved veterinary care will be performed at Virginia Commonwealth University before experimentation, to include environmental enrichment with toys. Animals will be housed in the animal facility at the Virginia Commonwealth University vivarium. All experiments will be conducted under IACUC and NIH guidelines and are approved by the animal care committee before experiments commence. Disposal of animal remains, pathological specimens, and animal bedding will be handled as required by the State mandated biomedical waste tracking act.

4. Ferrets will be euthanized with sodium pentobarbital overdose followed by exsanguination. Lungs will be inflated and preserved for histological examination.

LEADERSHIP PLAN

Because of the interdisciplinary nature of this proposal, a team-science organization has been selected with Dr. Longest and Dr. Hindle both serving as PI (i.e., a multiple PI model). Dr. Longest and Dr. Hindle will provide oversight of the entire Program including the development and implementation of all policies, procedures and processes. In these roles, Dr. Longest and Dr. Hindle will ensure that systems are in place to guarantee institutional compliance with US laws, DHHS and NIH policies including laboratory safety, information security, data and facilities. In general, Dr. Hindle will oversee the *in vitro* experimental aspects of the science and Dr. Longest will oversee the computational studies and device design work. Considering that the specific aims contain both experimental and computational aspects, Drs. Hindle and Longest will jointly be responsible for all specific aims. Based on expertise, Dr. Heise and Dr. Rubin will be responsible for the animal experiments in Aims 1 and 3, respectively. As the lead in device and airway model development, Dr. Longest will coordinate efforts with Dr. Willson and Dr. Rubin in terms of ensuring practical device application and efforts with Dr. Dodson in terms of securing the necessary airway CT scans and verifying that the selected scans are anatomically correct. Dr. Longest will serve as contact PI and will assume fiscal and administrative management responsibilities including maintaining communication with Dr. Hindle, Dr. Heise, Dr. Rubin and other key VCU personnel through already established monthly meetings. Meeting sites will rotate between the VCU Medical (Dr. Hindle) and Academic (Dr. Longest) Campuses. Summaries of these meetings (minutes, presentations, and study results) will be provided to all collaborators. To address fiscal distribution, sub-accounts will be issued to all faculty based on the original cost estimates at the time of proposal submission. Dr. Longest will be responsible for communication with NIH and submission of annual reports. Publication authorship and intellectual property distribution will be based on the relative scientific contributions of the investigators and other study personnel. If a conflict develops, the PIs will meet with the individuals concerned and attempt to resolve the dispute. It should be noted that Drs. Longest and Hindle have worked together over 10 years without any conflicts. Nonetheless, if they fail to resolve the dispute, the disagreement will be referred for resolution to a designated senior executive within the VCU Office of Integrity and Compliance who has the authority to settle the disagreement but who is not directly involved in the disagreement.

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Resource Sharing Plan

This project will develop formulations and devices for the effective delivery of aerosolized surfactants to the lungs of infants receiving various forms of noninvasive ventilation. Completion of project aims will result in (1) new highly dispersible dry powder formulations of lung surfactant consisting of submicrometer primary particles, (2) new aerosol generation devices to efficiently disperse dry powder surfactant formulations, (3) new methods and interface devices to efficiently deliver the surfactant formulations during noninvasive ventilation, and (4) new methods to target surfactant aerosol delivery to the alveolar region. In addition, aerosol efficacy will be tested *in vivo* in surfactant depletion animal models, and regional lung delivery efficiency will be evaluated and optimized using new **validated** human infant complete-airway computational fluid dynamics (CFD) models. The findings of these studies as well as the *in vivo*, and new numerical and *in vitro* models will be detailed in a series of journal papers. These manuscripts will be targeted to biomedical engineering, aerosol science, respiratory drug delivery, and medical journals. Furthermore, we agree to provide additional details and to answer questions related to our numerical models and experimental procedures for researchers interested in performing similar analyses or for clinical testing and application of the methods developed.

Authentication of Key Biological and/or Chemical Resources

The proposed program involves multiple key biological and chemical resources:

In vitro human infant airway models (nasal airway models for prong size evaluation): The models will be developed from pre-existing scans at Children's Hospital of Richmond from 5 infants in each of the following weight ranges: 1600 g, 2600 g, and 3350 g. The combination of weights and using a sample size of five infants will seek to address the range of variability observed in this patient population. These models will be constructed using in-house rapid prototyping as with models previously developed by our group and will continue to the trachea.

In vitro human infant airway models (nose-mouth-throat airway models for N2L delivery platform evaluation): Realistic infant nose-mouth-throat (NMT) models will be developed based on pre-existing scans of preterm (~1600 g) and full-term (~3550 g) infants available at Children's Hospital of Richmond. These models will be constructed using in-house rapid prototyping as with models previously developed by our group and will continue to the trachea. Selection of representative models will be made by a board certified otolaryngologist (Dr. Kelley Dodson). Our previous studies have used similar methodology to identify representative adult anatomies, quantified based on the nasal surface area-to-volume ratio (SA/V). A full description of the methodology and characteristics of the models is provided in Walenga et al., Variability in Nose-to-Lung Aerosol Delivery. J Aerosol Sci. 2014 Dec 1;78:11-29.

Commercial pharmacy sources (e.g. Cardinal Health) will be employed to purchase commercial lung surfactant formulations (Survanta, Curosurf, etc).

The excipients used in this study to produce EEG surfactant formulations will be of USP grade from commercial sources.

Comparison of surfactant proteins B and C following spray drying will be performed using LC-MS. Reference surfactant proteins B and C will be obtained extraction from the initial liquid commercial formulations. This methodology will be employed to detect changes from the starting material that may be induced during the spray drying process.