

Construction and Validation of an In Vitro Smoke Exposure System

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In Partial fulfillment of

Alaska Heart Institute Fellowship

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Abstract

To experimentally expose human respiratory cells to airborne pollutants, a controlled exposure system must be used. My project focused on designing, building and validating an integrated system for aerosol exposure specifically related to cigarette smoke. A previous design based on a 30 year old hydraulic driven Walton smoking machine and a prototype exposure chamber was used as a starting point. My final design consisted of three primary systems: smoke generation; smoke transport and exposure chambers; flow regulation. The building phase included ordering the components for the system, troubleshooting, and adjusting and assembling the exposure system. Upon generation by an InExpose aerosol generator, the smoke is pumped into a buffer chamber, and is pulled from there through the system, exposing cells housed within Vitrocell chambers. The smoke dosage is regulated by the puff volume set within the InExpose system, as well as a flow controller/ monitor which regulates the rate of smoke movement downstream of the buffer chamber. These control mechanisms allow for a constant and therefore predictable exposure. Air through the flow controller is filtered from smoke particulate to allow for steady, pure gas flow. Filtering allows the pump and flow controller to function properly without contamination, or risk of error to flow rates. I was able to establish a relationship between particulate concentration and rate of gas flow through the system with my findings using a computer controlled particulate sensor. This allowed me to establish a guideline for smoke exposure. The final result of my project was a completed cell exposure system which is calibrated and tested. My design worked as expected to establish an integrated system to expose cells to smoke or airborne pollutants in a controlled manner.

Introduction

It has long been known that cigarette smoke negatively affects respiratory cells, however research on the subject has been inconclusive. Understanding how and why smoke deteriorates lung cell tissue is critical for developing therapies to prevent this damage. Recent advances in technology have allowed for the development of controlled *in vitro* exposure systems for studying the affects of cigarette smoke on human respiratory epithelial cells. The use of an air-liquid interface exposure system for experimentation has been integral to the advancement of in vitro respiratory research to model *in vivo* conditions.

Working with an existing, but outdated design, I revised the design and constructed a new system, which integrated the latest technology into one fully functional laboratory system. Reviewing the previous design allowed for simple adjustment of key components and the addition of new units. Once the initial components were decided upon, orders were placed to various laboratory and equipment supply companies. Once items were received, assembly was required followed by calibration, and further testing. The system was tested for consistency and reliability as well as for the required dosing for accurate exposure. Using a computer controlled infra red particulate sensor, calibration and testing was done by adjusting the flow rate of the air and through the use of various cigarette types. The sensor provided unique and highly accurate data based on the amount of particulate present during each smoking situation.

Design and Construction

Smoke Generation: Initially, smoke is generated by a computer controlled system of a pump, valves, and rubber tubing routing system. This system was designed and supplied by InExpose (Montreal, Quebec, Canada). This system included computer software which controls the system, and monitors results. Mainstream smoke is pulled from a single burning cigarette by a small dual valve pump, and pumped through to a buffer chamber. The puff volume is controlled by varying the duration of the pump's pull. Once the desired puff volume is obtained, a valve closes off the route to the cigarette and a second valve opens a path for fresh air to be pulled into the system forcing any remaining smoke in the lines to be pushed into the buffer chamber. The smoke then rests

in the buffer chamber briefly, allowing for natural chemical changes to occur in the smoke, similar to a functioning lung. As smoke mixes with air, it undergoes chemical changes such as cooling, mixing and phase changes. The buffer chamber allows these natural alterations to occur, as they would in an airway. It is this smoke which is used to expose respiratory epithelial cells *in vitro*.

The smoke to be used in the exposure follows a path through a computer controlled infrared particulate sensor, and subsequently to the cell exposure chamber. This Infrared sensor is used to monitor and record levels of particulate matter found in

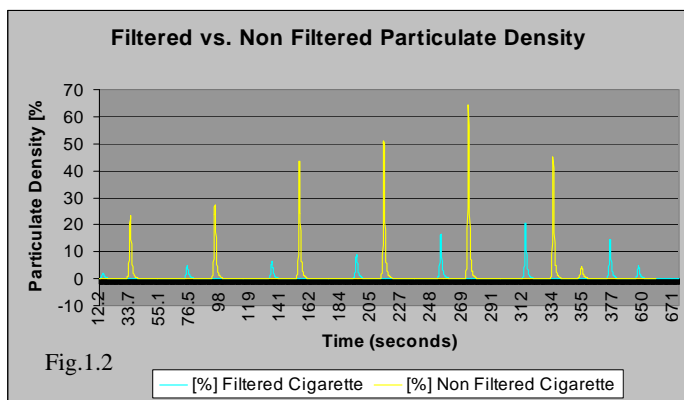


Fig. 1.2

the air passing through it. This sensor was integral to the system calibration and testing phases as it provides very accurate data and consistent readings of particulate levels. It was used to compare flow rate particulate, as well as various cigarette type equivalents.

Once the smoke has been held in the chamber, a valve opens downstream of the buffer chamber to allow for the extra smoke to be purged from the system until the next puff is taken. This valve allows the remaining smoke to be purged from the lines, buffer chamber, and pumps much like a smoker exhaling after holding the smoke in their lungs. This valve simulates a smoker taking the cigarette away from their mouth and breathing ‘fresh’ atmospheric air. This entire process is repeated throughout the duration of the cigarette’s burn life. As the initial smoke generation takes place, a secondary system is applied to use the generated smoke.

Exposure System: Downstream of the buffer chamber and purging valve, the smoke follows a path into the exposure unit (Vitrocell, Hanover, Germany). The smoke passes into the exposure unit containing three separate exposure chambers. It is in this unit that the cell exposure occurs. These chambers house the cultured cells, and keep them at a regulated optimal condition. By providing a warm and moist environment for the cells to be housed in during exposure, the cells undergo very little changes other than the

exposure itself. The smoke is then drawn out of the chambers and purged from the system.

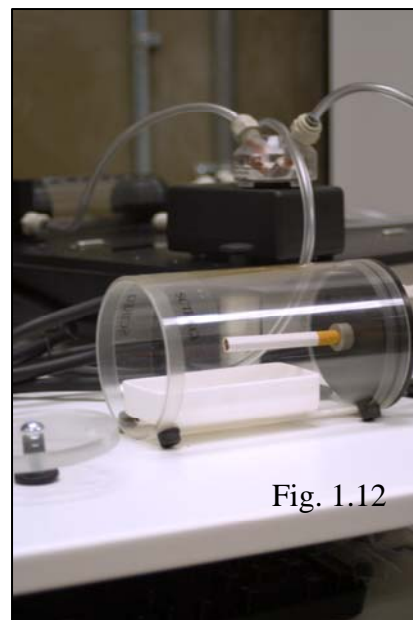
A secondary, constant pump, regulated by a flow controller/meter is used to create a vacuum effect which essentially pulls smoke from the buffer chamber in to and out of the exposure unit. This pump provides the force to move air from the buffer chamber to the rest of the integrated system. In order to control the rate of air movement, a flow controller/ meter is placed in line between the pump and exposure unit. This regulates the speed of smoke travel through the system. An inline filter protects the flow controller and secondary pump by removing any remaining particulate from the air. The secondary pump is integral to smoke transport; without it the smoke would not move from the buffer chamber into the exposure unit until the purge cycle. The flow controller is critical to the exposure system as it regulates the volume and rate of air movement, thus controlling the amount of smoke or air in the exposure unit.



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Troubleshooting: Assembly of the system mainly took place as components arrived. Once all parts arrived and final assembly was complete, a few issues were brought to light. Adapters were purchased and installed, allowing for all components to work in sync with one another.

The InExpose system was designed to purge out excess smoke at the end of one cycle through the exposure chambers, thus exposing the cells to all the smoke in an uncontrolled manner. The solution to this problem was to add another InExpose computer controlled 2-way valve set to close the pathway to the exposure chambers while opening a pathway to a purge line to let the excess smoke escape. This solved the problem and allowed for ‘exhaling’ to proceed in a controlled manner without influencing the cell exposure. Once



this problem was solved, the system functioned as needed as was ready for further testing.

Cigarettes: To choose which cigarettes to test, various online smokers' forums were consulted to determine popular brands. Cigarettes used included Marlboro Reds, Camel Filters, Marlboro Ultra Lights, and Newports, all varying slightly in tobacco content and filter length, thereby affecting smoke concentration levels. These choices make it possible to relate our data to current trends and social aspects of smoking.

Testing and Validation

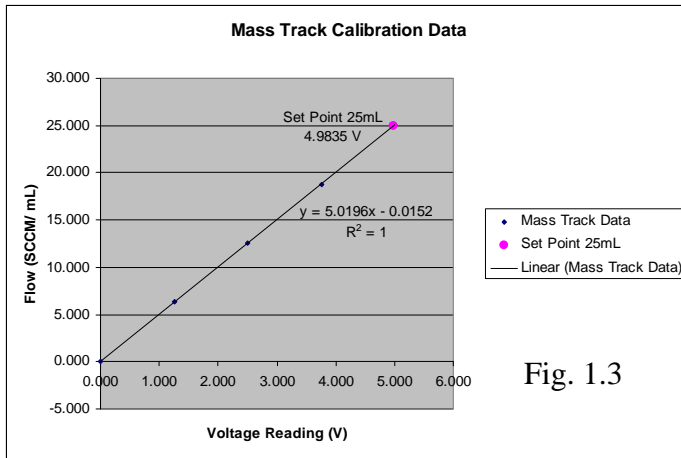


Fig. 1.3

Flow Control Calibration: An initial calibration of the flow control was performed. This was done using the factory supplied flow rate calibrations for the flow controller/meter. A regression was performed on that data and was then used to find the proper setting necessary to run the

system at the known equivalent flow rate. Previous testing in Dr. Knall's lab and by Vitrocell had established the optimum flow rate for exposure to be 25ml/min.

Cigarette Comparison: It was possible to compare the particulate content of smoke generated from various cigarette types using the completed system and the computer controlled particulate sensor. Particulate readings were taken from at least three subjects of each type of cigarette, graphed using Microsoft Excel software, and compared.

This comparison yielded interesting results. Although individual cigarettes within a type varied slightly in particulate content, each cigarette type showed a consistent pattern (Figs 1.4, 1.5 & 1.6). Clear differences in particulate concentration were seen across different cigarette types, regular, ultra light, and non-filtered. Ultra light cigarettes have considerably less particulate than their regular

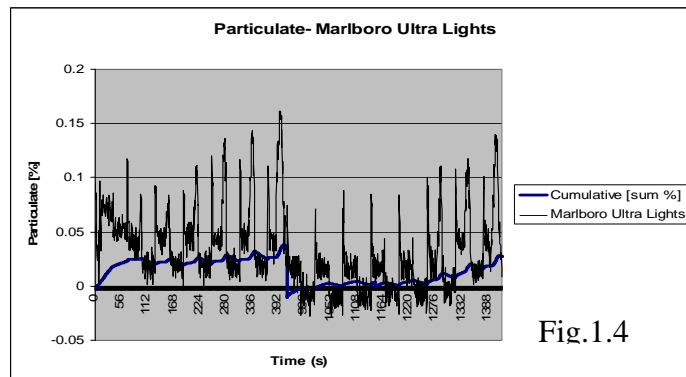


Fig.1.4

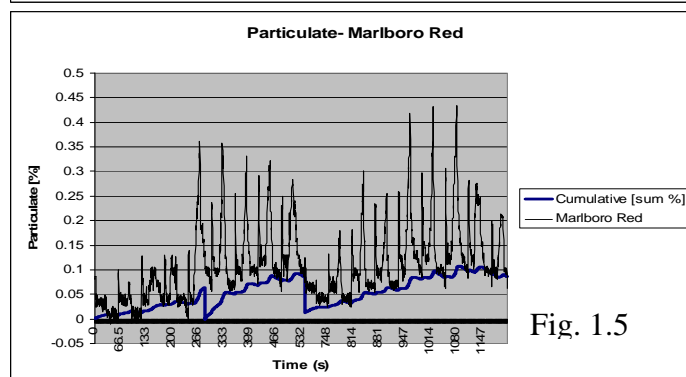


Fig. 1.5

counterparts (Figs 1.4 & 1.5). This difference might account for significant long term differences in health effects of regular versus ultra light cigarettes.

Through this testing for particulate, it also became clear that each cigarette had a common trend appearing in their burn cycles. As seen in fig. 1.5, each cigarette exhibited a peak in particulate at some point during the burn cycle, typically near the end. The reason behind this is unknown. It may be related to the burn chemistry in each cigarette. The results gained from this preliminary system use were integral to the success of the overall project. These results show that the system functions fully and as expected and is calibrated for full cell exposure.

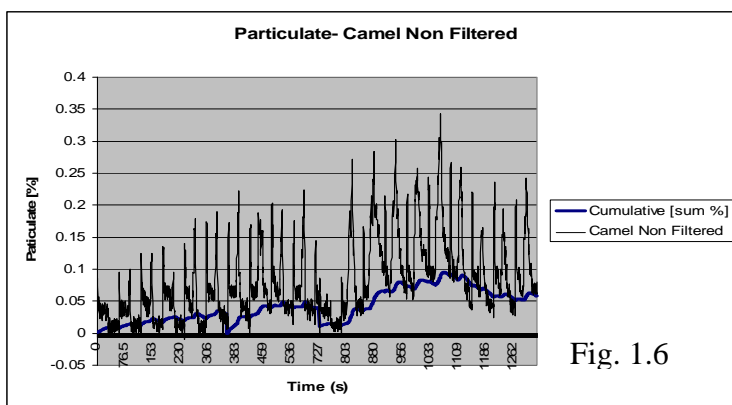


Fig. 1.6

A difference was seen in particulate content between filtered vs. non filtered cigarettes (Figs. 1.5 & 1.6). This raised the question of the purpose of the filter of a cigarette. Used and unused filters were dissected to

observe the change undergone in a burned filter. As seen in figure 1.7, the fibrous filters serve to capture much of the particulate. The difference between the filters show that filtered vs. non filtered cigarettes clearly contain different amounts of particulate. As this was to be expected, this further validates the working system.



Fig. 1.7