Develop a Microplate Accessory to Increase Oxygen Transfer Rates and Improve Bacterial Growth in 96-well Plates
(Technical Project)

Applying the Framework of Pacey's Triangle to Explain Microbial Variations Found Between Chinese and American Mothers During Pregnancy and Postpartum Stages
(STS Project)

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By
Nina Mei Brooks

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Technical Team Members:
Jared Mirt and Jake Thomas

On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

ADVISORS

Kent Wayland, Department of Engineering and Society

Jess Lee, Cerillo (LLC)
Sydney Decleene, Cerillo (LLC)
Daniel Carrier, Cerillo (LLC)

General Research Problem:

As new technologies allow for easier artificial growth of bacterial cultures, how can these advances be used to study and explain differences in the microbiomes of Chinese and American Mothers during pregnancy and postpartum?

A microbe is classified as a bacteria or fungi that produces either harmful or helpful effects to the human body on the microscale. The word bacteria often has a more negative than positive connotation, but less than one percent of all bacteria actually causes adverse effects on human health ("Bacterial Infections," n.d.). Rather it be through foods, such as yeast and cheese, or in vaccines, like the common flu shot, science has shown that microbes are an essential aspect of human existence. In 2021, the international market for microbial products was estimated at roughly 190 billion dollars. This market includes broths used to provide nutrients to bacteria, plastics and glassware that are used as environments for the bacteria, and machines used to collect data on how the bacteria grow. The microbial market value is expected to skyrocket to over 300 billion dollars in the next decade ("Microbial Products Market Size," n.d.).

The growth of this field is largely attributed to advances in microscopy and cell culture that have allowed scientists to better understand how to mimic conditions found inside of the human body to grow bacteria outside of the body. Manipulating these bacteria has become extremely adventitious to society, as researchers can now genetically modify and grow microorganisms by request. This increase in the ability to test and replicate bacteria has allowed scientists to create more effective vaccines and new classes of drugs.

While significant strides have been made in this field, it seems that one common tool used in bacterial cultures hasn't changed at all. The 96-well plate is a small plate made of clear plastic that contains 96 identical wells, each with a working volume around 300 microliters. The 96-well

plate is the most common way to grow bacteria, and the design has not been modified significantly since its' creation in 1951. The human body optimizes temperature, oxygen transfer, fluid evaporation, and light interference to maximize bacterial growth. Yet, these are all factors that have proven difficult to mimic when culturing bacteria artificially. So, growing bacteria at micro-scale volumes has been one solution in an attempt to control this plethora of factors. However, these microenvironments often prevent oxygen transfer within the wells, which leads to bacterial cell death. A new market for microplate accessories has been created in an attempt to find ways to introduce more oxygen into these small working volumes. The technical aspect of this paper will focus on creating an accessory to the 96-well plate to improve the oxygenation rate and consequent growth of bacterial cultures.

Shifting resources towards optimizing bacterial growth has caused a lot of attention to be drawn towards existing microbes in the body. The composition of the human microbiome, in particular, has piqued the interest of many. The success of bacterial growth and type of bacteria that thrive are based on conditions set by their host environment. It has been hypothesized that studying just the microbiome can predict the environmental conditions of where someone lives or what kind of influences can make their daily experiences different from someone else. Whether this is through air pollution, diversity of ingested foods, or access to clean drinking water, the microbiome changes based on what one is exposed to. In recent years, studies have specifically targeted the human microbiome at the earliest stage of life; the main subject of these studies is mothers. This STS portion of this project will focus on exploring why this microbiome has been found to differ in mothers from different parts of the globe.

Develop a Microplate Accessory to Increase Oxygen Transfer Rates and Improve Bacterial Growth in 96-well Plates:

What baffle geometry prevents optical density interference, increases bacterial growth, and fits universally with all 96-well plates?

Bacterial cultures are used in microbiological studies for a wide scope of applications. Pesticides, vaccines, and blood anticoagulants use bacteria to combat disease ("Bacteria and Humans," 2021). Bacterial concentration and growth patterns vary when in-vitro, outside the body, or in-vivo, inside the body, which make using them in biomedical applications, such as vaccine creation, a very long and complicated process. Spectrophotometry is used to quantify how much bacteria grows in a certain time frame, or essentially tells how successful the artificial culture was. In order to perform spectrophotometry, 96-well plates are placed into something called a microplate reader. A microplate reader is a device that measures bacterial growth through a measurement called optical density, which essentially determines how much light is transmitted through the sample. 96-well plates are slid into a slot on this device and then light is shown dowards through the wells.

The 96-well plate has some limitations, beginning with low oxygen transfer rates that disrupt bacterial growth. This is in part due to the inability to physically mix each well because it is so small and consequent stagnation of the well solutions. Therefore, 96-well plates are often placed on shakers. A shaker is a rotating, flat plate that sloshes solution within wells back and forth at a constant rate. Shakers have been shown to encourage culture disruption and introduce oxygen, but problems still arise. These 96-well plates, even on shakers, still can't grow bacteria as well as it naturally grows within the body. This is due to low oxygenation that the bacteria need to live. One solution has attempted to prompt oxygenation by placing the plate on a shaker and varying revolution and speed (Duetz et al., 2001, Bates et al., 2010). Other current solutions have moved beyond the shaker to redesigning the 96-well plate itself. It has been hypothesized that the

cylindrical shape of the wells does not promote mixing as well as sharp corners would. Therefore, some plates have been designed with rectangular wells or star-shaped geometries (Lattermann et al., 2014). Yet, both of these attempts have not resulted in any universal changes to the 96-well plate itself.

Thereby, this project aims to create a microplate accessory that will promote oxygenation through the introduction of baffles. This microplate accessory will be an addition to the 96-well plate. The proposed accessory for this capstone project is a lid that incorporates post-like extrusions into each individual well on the 96-well plate; these extrusions are called baffles. When using this accessory on a 96-well plate on a shaker, these baffles introduce unique structural changes within liquid bacterial solution that will promote culture disturbance and allow oxygen to enter the wells. Spectrophotometry will be performed in a microplate reader that will quantify the success of this project. With this in mind, these baffles need to prevent scattering and dispersion of light when it is applied to the top of the plate. If the baffles interfere with the light shown through each well, inaccurate readings of bacterial growth and optical density will ensue. Thereby another aim of this project is to create hollowed baffles that allow for light to directly shine vertically through each well for accurate optical density readings. To complete the technical portion of this thesis, direct collaboration with the Charlottesville-based company Cerillo LLC will be conducted.

Using the guidance and mentorship of the company, our team aims to design multiple 3D printed baffle designs using computer-assisted design (CAD) software. Several rounds of design will draw from literature, as well as scientific studies regarding bacterial growth on different mediums, substrates, and with exposure to different scaffolding geometries. Optical density tests will ensure that light refraction is minimal, and phase contrast microscopy will allow dispersion to be examined. Once a single baffle design is chosen as the most effective at dispersing material,

then E. coli bacteria will be used to test oxygen transfer. Spectrophotometers and orbital shakers will later be in use with the bacterial samples to ensure the livelihood of the samples. The effectiveness of the entire microplate lid will have to meet our set criteria regarding oxygen transfer rate (OTR), bacterial dispersion, force, light interference, and volume loss by evaporation. The marginal acceptable values for these will be determined using literature.

Unraveling the Human Microbiome to Understand Deeper Societal Differences Experienced by Mothers Transnationally: *How can we examine the microbiome of Chinese and American mothers to explain cultural, organizational, and technical differences between both societies?*

A list detailing the concentrations of bacteria found in the human gut can tell more about a person than one thinks. Bacteria serves as a crucial determinant for the amount of nutrients that the body is absorbing, the environmental factors the body is breathing in, and potentially even explain if you were born via cesarean section or if you were breastfeed as a baby (D'Argenio, 2018). Our external exposures play an essential role in developing biodiversity within our bodies. In these ways, we can see that there is much more at play than our genetic makeup when we consider bacterial and immune health. Our environments, social positions, economic status, and even racial demographic have suggested that our microbiomes are adapting to our social conditions and experiences.

The microbiome of Chinese mothers has been proven to be different than in American mothers (Zhang et al., 2022, Niu et al., 2020). Researchers cannot find scientific explanations for why these differences occur between different countries, but through sociological analysis it has been predicted that society views consumption as a main factor (Gupta et al., 2017). Consumption of resources, and consumption of products all create different effects on lifestyle. Those in hunter-

gatherer lifestyles usually live in smaller dwellings may be less open to modern medicine that one would find in a large city during pregnancy and less likely to have access to them. very regularly. However, those in urban populations can be exposed to the newest types of medicines and drugs. Liu et al., even cite the use of topical creams or contraceptives as a possible reason for microbial differences in Chinese versus American women. I am to examine Chinese and American women living in rural areas and what types of treatments they receive during pregnancy and why they choose to accept or reject certain medical amenities.

To better understand what socio-technical factors contribute to differences in microbiomes across countries, I plan to conduct background research on societal views on different types of pregnancy ailments used in China and America via editorials and news articles. I plan to use Pacey's triangle to explain the organization aspect, such as pharmaceutical companies, technical aspects, such as modern medicine versus traditional medicine, and the cultural aspect of each country. At an organizational level, I will examine the access of healthcare given to mothers in each country, such as usage rates in rural areas. Population surveys and documentaries will also aid my discovery of public opinions on these changes in both countries. It has been shown that methods of birth can alter the microbiome, and I aim to discover if there is one popular method of delivery or multiple accessible ways to give birth in China and America (Niu et al., 2020).

This topic is important in that racial disparities in healthcare, especially with women, are often ignored. Many test subjects for clinical trials are often White and male, thereby making results of studies extremely skewed and ignorant of genetic differences found between sexes and races. This paper aims to explore how society and technology has changed the way childbirth is perceived in China and the United States due to preexisting cultural and organizational differences held by women in each respective country.

Ties Between Increasing the Success of Bacterial Studies and Understanding Just Microbiomes Have Been Found to Differ Amongst Mothers of Different Nationalities: *How can growing bacteria lead us to understand how cultural, organizational, and technical differences between the U.S. and China alter human microbiomes formed at birth?*

I hope with the creation of the microplate accessory, bacteria growth can be accomplished by increasing mixing and oxygenation within the individual wells. These studies regarding bacteria will ideally provide explanations for foundational differences starting at the earliest stages of life. Devices, such as the 96-well plate, have allowed culturing of specific cell populations in-vitro to occur. The ability to manipulate such populations has prompted a deeper delve into studying the human microbiome. With this project, I hope to better correlate cultural, organization, and technical differences between China and the United States regarding the use of traditional or modern medicine during pregnancy and how it has altered societal views on health and affected immune health. Through researching different popular opinions and views of childbirth at a population and governmental level, I hope to see what effects this can have on future populations.

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