Voxelated 3D Bioprinting Highly Organized Yet Heterogeneous Tissue Constructs

And

The Rise Of Anti-Resistant Bacteria

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Technical Project by Leander Nguyen

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

Signature ____ Date __________
Approved ____ Date __________ , Department
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Introduction

Bioprinting is an emerging field where 3D printing technology is utilized to produce tissue and organs similar to natural human body parts(*BIOPRINTING | Definition in the Cambridge English Dictionary*, n.d.). Through the use of an additive manufacturing process, where biomaterials such as growth factors and cells are combined, 3D tissue constructs can be made to mimic the micro and macro environment of human tissue(Khademhosseini et al., 2006). This can potentially reduce the need for organ donors/transplants(Vijayavenkataraman et al., 2018) and be used for drug testing and clinical trials. Although there have been established methods (extrusion-based printing, inkjet printing, and stereolithography) that have had varying degrees of success in creating tissue constructs, there are limitations in controlling cell distribution and density due to the variability in bulk printing and difficulty in reaching deep regions of the construct due to small pores(Heinrich et al., 2019). This has led to challenges in building a construct that can fully mimic the highly structured microenvironment of tissue that would normally be seen *in vivo*(Gillispie et al., 2020). Nearly all extrusion based 3-D printing methods print 1-D filaments, which are then stacked to create 3-D tissue constructs. However, this leads to a lack of porosity and thus limits nutrient transport essential for cell growth.

One ethical/societal issue that may rise as bioprinting evolves and advances, is the risk of widening the socioeconomic gap in medical treatments. Currently, new technology and personalized therapies, such as gene therapy and cancer treatments are expensive and are not widely accessible to the general public. 3D bioprinting may run into the same problem of only being accessible by people who can afford it. However, the technical subject of the STS prospectus and the technical topic for the Dept. of Biomedical Engineering is **not related**

Antibiotics are medicines that are used to treat once deemed incurable bacterial infections. They have saved the lives of millions, but are in danger of being obsolete. Currently, the rise of antimicrobial resistance (AMR) is occurring throughout the world and if not properly addressed, may leave us in a worse state than before the invention of antibiotics. There are a variety of reasons that have led to the abuse and inappropriate use of antibiotics that will be discussed in depth later in this paper.

Technical Topic: Voxelated 3D Bioprinting Highly Organized Yet Heterogeneous Tissue Constructs

3D bioprinting is emerging as a novel and promising method for the biofabrication of complex constructs that can mimic native tissue, with far-reaching applications in tissue engineering and regenerative medicine. Currently, organ availability is one of the main challenges that limits transplantation in the United States. Over 100,000 patients need organ transplants and more than 7,000 deaths occur annually while patients are waiting(Merola et al., 2016). In addition, even with state-of-the-art immunosuppressive drugs, most organ transplants slowly degrade due to chronic immune rejection, thus leading to long term failure(Kloc & Ghobrial, 2014). Using 3D bioprinting, organs can be manufactured using bioink containing living cells that are derived from the patient, mitigating rejection through greater biocompatibility and personalizing medical care(Sigaux et al., 2019). Additionally, drug screening for pharmaceutical development, disease modeling and testing, and other *in vitro* applications would also benefit greatly from biofabricated tissue models. Compared with *in vitro* testing, animal tests range from 1.5 to 30 times more expensive and experiments can last years before being approved by the FDA(Van Norman, 2019). Precisely manufactured tissue mimics made of human cell types will produce models that more closely reflect the microenvironment of human tissue, compared to animal or synthetic biomaterial models. This will bring about more accurate, cost efficient, and time efficient results from experimentation and testing that can be translated into medical applications.

Even with the evolution and significant improvements in 3D bioprinting methods over the past years, there are still many limitations to producing precise and controlled tissue constructs with heterogeneous and hierarchical complexity. Exploring the use of spherical hydrogel particles as 0D voxels will lead to a greater control of porosity throughout the construct and establish innovative and effective 3D bioprinting techniques. Specifically, this technique will examine how to precisely control the level of tissue porosity through varying sizes of 0D voxels, and determining the effects on nutrient transport throughout the tissue construct. If the aims proposed within this capstone project are successful, it will prove the efficacy of using 0D voxels to create highly structured and porous 3D tissue constructs that promote the migration and proliferation of seeded cells. In the future with more research being done, it may be possible to create highly complex organs using voxelated 3D bioprinting. Creating viable organ constructs *in vitro* will help address challenges associated with organ donor transplant shortages and has the potential to save countless lives.

Innovation:

Through this project, a novel and unique method for 3D bioprinting tissue constructs will be further studied. Conventional bioprinting methods employ a layer-by-layer method to construct a 3D structure, using 1D filaments as building blocks(Dey & Ozbolat, 2020). With the use of spherical 0D voxels, which are the basic building blocks of 3D structures, a higher order of precision and control can be achieved in constructing a porous microenvironment of the tissue. Unlike 1D filaments, 0D voxel particles can be precisely sized and distanced to produce the optimal porous microenvironment for tissue development.

Current State of the Art

Current methods for 3D bioprinting include inkjet, extrusion-based, stereolithography, and laser-assisted printing(Li et al., 2016). These methods pose limitations surrounding the level of complexity that can be achieved and the precise positioning of cells within the constructs. Extrusion based 3D printing has several limitations such as inadequate control, introduction of shear stress to cells, and only being applicable for certain viscous bioinks(Derakhshanfar et al., 2018). Although the cell viability of extrusion-based 3D printing is noted to be relatively high (89%), the limitations of printing with 1D filaments make it difficult to create complex, structured tissue microenvironments(Derakhshanfar et al., 2018). Inkjet bioprinting has an inherent inability to provide a continuous flow, thus lacking precision in droplet placement/size when compared to other printing methods. The cell viability (40-80%) and density of inkjet printing methods are also low due to shear and thermal stresses, which is a major limitation to creating highly organized tissue constructs that promote cell proliferation and migration(Li et al., 2016). Stereolithography, although having a documented cell viability of over 90%, has numerous limitations, including the risk of damaging cells through the use of UV light, potential harmful cytotoxic effects of the photo initiators, and the inability to remove the supporting structure within the tissue construct(Li et al., 2016). Laser assisted bioprinting is costly, time consuming when preparing the ribbon layer, and suffers from low stability and scalability. These limitations make clear the need for a novel bioprinting solution that achieves high cell viability levels without compromising the complexity and functionality of the microenvironment structure(Li et al., 2016).

Novelty of Proposed Method

The research lab at UVA, led by Professor Liheng Cai, has technically modified a conventional mechanical extrusion-based microfluidic printhead to be able to print hydrogel particles, using a motorized syringe pump, thus engineering an innovative new 3D bioprinter. Conceptually, the digital assembly of spherical particles produces a novel method for customizing diameter and distance between hydrogel particles to print tightly controlled and organized tissue constructs. Not only this, but the diameters of the particles can be dynamically modified during the printing process, allowing for heterogeneous structures, which cannot be accomplished with current 1D filament based bioprinting. Though current methods, such as extrusion-based or inkjet technologies, have been able to print heterogeneous tissue constructs containing multiple cell types, they still employ a layer-by-layer technique that cannot fully recreate the cellular and extracellular organization and complexity of native tissue(Ashammakhi et al., 2019). Whereas 1D filaments allow for the construction of discrete zones of different cell types of biomaterials, the variable size and greater degrees of freedom of voxels enables the design of tissue constructs at higher printing resolutions, down to variations between single microparticles(Placone et al., 2020). Initial studies confirmed cytocompatibility of encapsulated bacteria and mammalian cells in the hydrogel particles arranged in 3D lattice structures. Our capstone project will be focusing on augmenting the porosity of these 3D structures, using variable sizes and distances of voxel particles, to study nutrient transport, and thereby further support the improvements to 3D bioprinting made by this novel method.

STS Prospectus

Introduction:

Before antibiotics, infections such as pneumonia and diarrhea, were the number one cause of death in the world. The average life expectancy of a person in the United States was 47 years. After penicillin was accidentally discovered, it was mass produced for the general public. This sparked the antibiotic golden era, which led to the discovery of many more antibiotics over the years. This revolutionized how the countries all over the world treated infectious diseases that were once deemed incurable. In the United States for example, the average life expectancy rose to 78.8 years with the population of older people increasing from 4% to 13%(Adedeji, 2016). However, over time antimicrobial resistance (AMR) has developed and is one of the greatest threats to mankind. According to the CDC, the United States alone currently prescribes over 270.2 million antibiotics per year; around 1/3 of which are either unnecessary or inappropriately prescribed(CDC, 2020). This overuse of antibiotics can promote the birth of resistant bacteria, thus causing severe infections, longer hospital stays, and increased mortality rates. In the 2019 antibiotic resistance threat report, more than 2.8 million antibiotic resistance infections occurred, causing approximately 35000 people to die(CDC, 2020). Currently, resistance has been seen by nearly all antibiotics that have been produced(Ventola, 2015). If the misuse of antibiotics continues, then in the foreseeable future there may emerge an untreatable bacterial infection/disease that will be incurable and devastating to the general public.

There are many economic, social/cultural, and political aspects that contribute to the problem of increased AMR all over the world. As mentioned in the introduction, most antibiotics are prescribed through a general physician, around 30% of which according to the CDC are unnecessary and inappropriately prescribed(*Overuse and Overprescribing of Antibiotics*, n.d.). This is due to the inherent nature of the patient-doctor relationship/culture. When patients go to the doctor, they expect to feel better. Because antibiotics were seen as a miracle drug that treats illnesses that were once deemed incurable, many patients ask for it even when they won't help. For example, a patient suffering from a cold, bronchitis, or upper respiratory tract infections will often seek antibiotics for a speedy recovery(Fletcher-Lartey et al., 2016). This expectation from the patient results in the doctor feeling pressured to prescribe antibiotics inappropriately, as they don't want to risk losing the patient. There are also many economic aspects that further complicate the problem of AMR. Currently, there is no viable antibiotic market as developing them is a complex and risky process. This leads to insufficient funding in the later stages of development which is coined the "Valley of Death". In addition, the profit made from antibiotics is not enough to cover the cost of development, and has caused multiple pharmaceutical companies specializing in antibiotics to file for bankruptcy(Gupta & Nayak, 2014). As a result of the profit driven mentality of pharmaceutical companies, only two new classes of antibiotics have been created in the past three decades. Furthermore, in poorer countries such as India and in some low-income areas in China, antibiotics can be obtained easily by purchasing them over the counter(Morgan et al., 2011). One reason may be that low income countries lack the necessary funds to coordinate an effort to establish rules and regulations regarding the usage of antibiotics. Political aspects also help contribute to the problem of AMR. In countries such as the United States and China, there are numerous private corporations/hospitals that provide healthcare to the common public(Angell, 2008). This allows the private hospitals to have their own rules and regulations regarding the usage and management of antibiotics due to there being no centralized government establishing order. However, Sweden is a current example of a government centralized system. Most of the hospitals in Sweden are public and are mainly government funded, thus making it easy to regulate and establish rules. As a result of this centralization and coordination, Sweden is one of the countries with the lowest antibiotic use and has worked to decrease the usage of antibiotics since the mid 1990's. Overall the problem of AMR is extremely complex due to involving many different aspects and moving parts. Compounded with the fact that resistance to new antibiotics will mostly likely occur in the future, AMR will always be something humanity has to tread carefully on.

Research Question:

For my STS prospectus, I will be conducting an in depth comparative analysis of the rise of AMR in the three different countries mentioned in the previous paragraph(China, United States, and Sweden). AMR is a global problem, but the reason contributing to it depends and can vary depending on the circumstances. I am proposing three research questions that will be applied to each country. The first is how does the patient-doctor relationship contribute to the problem of antibiotics prescription and AMR? The second research is how the current government involvement/policies of antibiotic regulation impacts the growth of AMR. The last research question is how the profit driven mentality of pharmaceutical companies, their business, and innovation strategies lead to the overdose and over prescription of antibiotics?

Literature Review:

Before antibiotics, infections such as pneumonia and diarrhea were the number one cause of death in the world. This revolutionized how countries all over the world treated infectious diseases that were once deemed incurable. However, over the years the increased usage of antibiotics all over the world has led to the rise of resistant bacteria. If precautionary measures are not taken, then in the not so distant future there may be an infectious disease that is incurable due to being resistant to current antibiotics. The following literature review discusses social, economic, and political factors in the United States, China, and Sweden that help contribute to the rise of antimicrobial resistance (AMR) and certain solutions that are being implemented to help combat it.

Sanchez and Demain discuss the rise of antibiotic-resistant bacteria(Sánchez & Demain, 2015). They found that bacterial infections even with the use of current antibiotics are still causing thousands of deaths and billions of dollars in healthcare costs. They also found that 70% of infections within the United States are now resistant to at least one type of antibiotic that is currently used in hospitals. The lack of new and innovative drugs is also mentioned. In 1996 the FDA approved over 120 drugs, which dropped greatly to 20 in 2005. The number of new and approved antibiotics is even less; in 2004 only 1.6% of the compounds in clinical development were antibiotics. Sanzhez and Demain explained that this was due to primarily a couple of reasons. The first is the merger of many pharmaceutical companies, such as Wyeth and Pfizer, which decreases the number of companies searching for new antibiotics. The second reason is that discovering new natural products with antibiotic activity is difficult as most of it has already been discovered. The third is the increased cost and time of getting a new antibiotic in the market, which is around 1 billion dollars and 10 years respectively. Cooper discusses the need for government change to make the innovation for new antibiotics viable (Cooper & Shlaes, 2011). Cooper mentions that the cost of antibiotic phase III trials alone cost an alarming 70 million dollars and that current government grants and other venture capitals cannot fund this. Solutions have been debated over the past, but no concrete action has occurred. Also, because the FDA has demanded more costly studies to prove the superiority of a new antibiotic over a preexisting one, many companies have taken their work overseas. Doripenem is a current antibiotic that is used all over the world except for the United States. Cooper mentions that leadership and government intervention is required to change not only the strict rules of the FDA, but to also provide incentives to the pharmaceutical companies creating new antibiotics.

Harrison discusses how AMR is one of the greatest threats to humanity, but the magnitude and impact are still deeply unknown(Harrison et al., 1998). Harrison mentions how no country, including the United States, has a reliable antimicrobial resistance surveillance program to monitor trends in antimicrobial usage. Multiple surveillance groups around the world are attempting to create systems to do this, but are uncoordinated and unstandardized. To identify the problem and magnitude of antimicrobial resistance, coordination on a global level needs to occur to ensure that humanity can adequately prepare for the inevitable problem of antimicrobial resistance.

Gröndal also discusses the management of antibiotics in medical practices and how it contributes to the problem of antimicrobial resistance(Gröndal & Holmberg, 2020). Gröndal mentions how clinicians and doctors want to be the gatekeepers and to have the authority regarding prescribing drugs without pressure from outside sources such as the government. As a result, he discusses several studies that suggest physicians sometimes prescribe drugs for non-medical reasons to maintain authority. In addition, patients also see antibiotics as a miracle drug that cures everything and often ask for it when ill. As a result, Gröndal found that some general practitioners prescribe antibiotics to simply keep the patient happy and to maintain loyalty. Gröndal's findings were replicated by Butler who conducted a qualitative study with structured interviews to better understand why general physicians were prescribing antibiotics for sore throats despite knowing that they will not help (Butler et al., 1998). Butler found that almost all physicians acknowledged that antibiotics were prescribed too often for upper respiratory tract infections. Most doctors however felt that the possible patient benefit outweighed the community risk of antimicrobial resistance, as their main priority was the well-being of the patient. In addition, Butler found that most doctors did not want to jeopardize their relationship with the patient and cited that if they didn't prescribe antibiotics, the patients would find another doctor who would. The doctors also feared medicolegal problems if the patient did get sick and being perceived as someone who did nothing for their patient. Overall, the studies by Butler and Gröndal show that social aspects between the doctor and the patient greatly influence whether an antibiotic is prescribed.

There are also the social/economic factors investigated that influence acquisition of antibiotics without prescription in India(Saradamma et al., 2000). Saradamma found that several conditions in India help facilitate the inappropriate use of antibiotics. The first is the government encouraging the expansion of pharmaceutical companies, thus resulting in more licensed pharmaceutical manufacturing units than primary health centers (20,000 vs 17,000). In addition, because of economic restraints and the perception that antibiotics were seen as a "miracle drug", it led to many people of low-income status to not consult a private practitioner, and instead to self-medicate. Saradamma discussed how many of these antibiotics were easily bought over the counter and often taken in inadequately. Only around 11% of people were taking the correct dosage and for the correct amount of time. Through the usage of logistic regression analysis, Saradamma found that the people least likely to follow this practice were educated and from higher-income families, suggesting that education can be a possible solution to reduce the inappropriate usage of antibiotics

As mentioned in the previous paragraphs, there have been increasing numbers of bacterial infections that have become difficult, and sometimes impossible to treat with current antibiotics. The United States federal government in response to this global threat has created a 5-year plan that was created in 2015. In this study, the GAO was tasked to examine the efforts and efficacy of this plan("Antibiotic Resistance," 2020). In this report, the GAO found that there were challenges regarding the surveillance of antibiotic resistance bacteria by the CDC, appropriate antibiotic use, and the development of new and innovative antibiotics in pharmaceutical companies. The GAO concluded their study by recommending multiple things that could be done to reduce the impact of antimicrobial resistance. This included things such as suggesting higher market incentives from the government to pharmaceutical companies producing new antibiotics and proposing that all healthcare facilities are required to implement stewardship programs. This would ensure that patients are receiving the right antibiotic at the right time, in the right dose, and for the correct duration.

Despite the problem of resistant bacteria rising in countries such as China and the United States, Sweden has the lowest levels of antibiotic use and resistance among the European Union. Mölstad discusses the strategies that Sweden is incorporating to successfully handle the rise of antimicrobial resistance(Mölstad et al., 2017). Due to an outbreak of penicillin-resistant pneumococci among children in the 1990's government authorities and professional organizations, such as STRAMA, have been working together to address the problem of AMR. Not only has Sweden passed legislation banning the use of antibiotics in agriculture, but they also are committed on both a national and local level to educate and monitor antibiotic prescribing. Moreover, Mölstad mentions how the government is responsible for the overall policy of healthcare, thus allowing for stricter regulations on the usage of antibiotics. In addition, the general public of Sweden seems to be well educated about the dangers of the overuse of antibiotics thanks to organizations such as STRAMA. André examined the level of knowledge regarding antibiotic treatment and awareness of antibiotic resistance in Sweden (André et al., 2010). Through cross-sectional interview studies based on a structured questionnaire, André found that 80.7% of people in the study agreed that bacteria could potentially become resistant to antibiotics and that most people trusted the doctor to make the correct decision for them.

Although the problem of AMR is inevitable some things can be done to slow down the usage of antibiotics. Ka examined the impact of an antibiotics stewardship program (ASP) in a hospital in Hong Kong(Ka et al., 2007). Ka found that before the implementation of the ASP, broad-spectrum antibiotics were prescribed inappropriately 28.9% of the time. However, after post-intervention, this decreased greatly, along with the overall consumption of antibiotics in general. In addition, Ka found that even though the ASP placed more regulations on the usage of antibiotics the quality of medical care was not jeopardized. Moreover, an economic analysis was done which showed that the implementation of the ASP would cost $71294 per year, but would save the hospital in Hong Kong $380899 per year.

In conclusion, the problem of AMR is inevitable and highly complex due to there being many social, economic, and political aspects contributing to it. However, each country has their own circumstances and problems that need to be fixed. In the United States and China for example they mostly use private healthcare, thus making it harder to establish regulations. (Angell, 2008). Although Sweden has taken many successful steps to combat AMR, they much like the rest of the world, struggle with the fact that antibiotics are finite. Fewer and fewer companies are attempting to innovate new antibiotics due to the whole process being so costly. Although the problem of AMR will exist for as long as humanity lives, this literature review shows how different countries struggle with different problems in regards to AMR, and the varying solutions that can be implemented to slow down the rise of AMR.

Research Method:

Two STS frameworks were mainly used to conduct my research. To start, I adopted Winner's perspective to determine whether or not there was politics underneath the design, production, and distribution of antibiotics. Through this, I was able to identify how the lack of government involvement regarding regulations, the social relationships between the patient and the doctor, and the profit driven mentality of pharmaceutical companies all help contribute towards the problem of AMR. I also applied LTS analysis to each of the three countries previously mentioned. This helped me better understand who exactly the system builders are in each country and how the system was built in the first place. Through this analysis, I was able to understand how the government, the doctors/patients, and the pharmaceutical companies are the major contributors. I also used LTS analysis to determine if there were any forms of technology that are going through a similar process of development. Overall, both of these frameworks helped me answer the research questions proposed earlier in the paper, by giving me insight into the political/social factors behind the production, distribution, and usage of antibiotics.

The research methods I used for this literature review were composed of books, studies, and research papers found from Google Scholar and from the UVA Virgo database. For the material that discussed the social aspects that lead to the rise of antibiotics, the authors mostly interviewed clinicians/general practitioners. This allowed them to better understand the relationship between the doctor and the patient. The papers discussing the political aspects mostly used data from other cited research papers to back their claim and studies to show how current government intervention can be improved. For the articles discussing the economics regarding AMR, they not only conducted studies to determine the efficacy of things such as the stewardship program, but also used data from other research papers to come up with legitimate claims and conclusions. An example of this was when Mölstad used data gathered from the Swedish government and other research articles to discuss how Sweden is one of the countries with lowest rate of resistant infections and how the rest of the world can learn from them.

Timeline:

The next step in my research plan is to do a deeper dive into literature looking to find more information and examples regarding the rise of antibiotic resistance and expand on what I have learned through my research. I would like to find more concrete examples and studies that help address my three main research questions in all three countries. Specifically, my goal by the end of the semester is to find five to seven solid sources that will directly help answer the research questions. I would also like to expand my research methods and to conduct interviews with doctors from charlottesville and other family members who work in hospitals if possible. This would allow me to have greater insight into the relationship between the doctor and the patient.

Conclusion:

The main goal and expected outcome of this STS prospectus was to gain a better understanding of the rise of AMR and to identify the system builders that were directly contributing to it in varying countries. Through my research, I discovered that there are many underlying social/cultural, political, and economical aspects that heavily influence the distribution, production, and usage of antibiotics. I also learned that different countries have different contributors as to why the problem of AMR is rising. Overall, AMR is one of the greatest public health challenges and requires effort on a global scale to slow down this inevitable threat. It is my hope that the main contribution of this STS prospectus is to reveal some of the main reasons behind the rise of AMR, so that in the future these problems can be properly addressed, thus potentially saving millions of lives.

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