The Best of The Journal of Young Investigators 2020
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Who are we and what do we do?

The Journal of Young Investigators (JYI) is a non-profit (501c3), independent, peer-reviewed journal that is run by undergraduates from around the world. As the only international journal of its kind, JYI aims to provide all undergraduates with training in scientific writing and publication opportunities.

We are a community of future scientists, writers, physicians, among many other things. We believe in providing a hands-on approach to provide all students, regardless of their background, with the resources for a successful career in science communication. To do this, we involve undergraduates in every step of the writing, editing, and peer-review process. Recently, we have spearheaded an initiative to provide undergraduates with resources for professional development, such as our webinar series. Furthermore, we run an online blog to allow undergraduates, as well as our advisory board, to more informally describe personal experiences and advice for a career in the sciences. Our advisory board is made up of graduated ex-JYI members who advise current members throughout the scientific writing and editing process.

JYI’s mission is to enhance science education for all students, regardless of location or background. As such, JYI focuses on the latter part of the research process: research communication, reviewing and being reviewed by peers, and publishing.

Most importantly, we believe that science communication should be a global effort built on collaboration and cooperation. 

“I joined JYI when I was in the second year of my undergraduate degree as a Science Journalist. I have since been a News and Careers Editor, Managing Editor, and Editor in Chief. I have gained invaluable skills throughout my time with the journal and have made friends for life. I am now the editor of a UK veterinary magazine and would not have the job I have now if it weren’t for the skills I gained throughout my time at JYI. I would wholeheartedly recommend JYI to any undergraduate looking to gain experiences outside of university.

-Amelia Powell | Former Editor-in-Chief

We also believe in a rich experience which brings with it meaningful relationships.

“As a Layout Designer, I remember how rewarding it was to see the papers that I worked on being released every month. Even now, years later, I make sure I read every paper, article, and blog JYI publishes. Seeing the hard work from the talented JYI staff makes my day every time.

I also remember meeting with the Executive Board (back when I was CTO) in Washington, DC. After a day of working together on different projects, presentations, and meetings, we went out

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to see the city. Our then SRE was super excited to see the National Mall. At the Smithsonian, I remember searching for the Narwhal, which our then ME wanted to see. And I remember taking a million photos at each in-person Executive Board meeting I was a part of (much to the chagrin of the others).

I made some of my closest friends while a part of JYI. These are just a few of the moments from our meetings and phone calls that stick with me. And every month I get to see JYI becoming even better.

"-Adam Sychla | Board of Directors

We pride ourselves in the opportunities that come with JYI involvement.

"I would say that personally JYI is the single best thing that I have done as an undergraduate in terms of promoting not only my understanding of academic writing and editing but also the scientific process as a whole. My role as an Associate Editor has opened the door to many amazing opportunities both inside and outside of JYI. For example, becoming a Research Assistant on an international expedition and taking up a Research Project with the British Geological Survey.

I accompanied a lecturer (who is actually my advisor for JYI) from my university department on a research expedition to Maliau Basin in Borneo, Malaysia. For this I successfully applied for my own research grant funding to become an undergraduate Research Assistant. We teamed up with a colleague from Germany as well as a Research Assistant from the University of Malaysia, Sabah to undertake a Paleolimnological study of a very remote lake site (Lake Linumunsut) within Maliau Basin Protected Area. Paleolimnology uses the law of superposition (that older sediment will be the deepest) to reconstruct climate, pollution, vegetation and other environmental records from inland water bodies. Our aim at Linumunsut was to reconstruct a local climate record for the previous 100 years (instrumental records only go back to 1990), to test whether long-range atmospheric pollution was impacting this remote protected area and to try to reconstruct the formation of this lake site, a unique landform in the area (being the only lake in the reserve). This involved much preparation and a challenging five day trek through a largely untouched rainforest with all of our equipment (including a sediment coring device and weights) just to reach the site. Whilst in Malaysia, I also helped out at a Paleolimnological Summer School at the University of Malaysia Sabah with the aim to inspire a new generation of Paleolimnologists. At the summer school I actually presented a short talk about what we do at the Journal of Young Investigators and how students can get involved.

"-Brittany Pugh | Former Associate Editor

JYI publishes research and review articles in the biological and biomedical sciences, physical sciences and mathematics, engineering and applied sciences, and psychology and social sciences. To find out more, and for opportunities to join JYI, visit our website at www.jyi.org.
Letter from the Editor

Dear reader,

Best of JYI is our annual print edition which showcases some of the work which impressed the JYI executive board and board of directors the most. This year we have quite a spread of papers for you from the effects of pets on urban wildlife to prosthetic motor clutches.

I found out some time ago that the first issue of JYI was published on the day I was born - a fact that really hit home just what a privilege it has been to direct the journal in a small segment of its 23-year-old history. In this time JYI has changed a great deal. Indeed, even over the past three years that I have in some capacity led the journal, I have seen it change and morph. We have begun translating our press releases and are in the process of getting indexed in academic repositories.

Moreover, like every organisation we have had to adapt to the small issue of a global pandemic. Part of this included running our first JYI conference, which starred a Nobel prize winner as speaker alongside undergraduates presenting their research. Given that the current leadership have, for the most part, never seen each other in the flesh, I find it somewhat moving how tightly knit teams can still form.

JYI is not only the largest and oldest undergraduate scientific journal, but it also represents an anomaly in academic publishing. As academia moves towards a model of “open science”, we represent a large, open-access and entirely free journal run entirely by dedicated volunteers. As my time as editor in chief draws to a close, I can only say that I am proud of all the work we have done. I sincerely hope you enjoy reading.

Best wishes,

Alexis Gkantiragas

Editor-in-Chief

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The FAC(t)S of Detecting T Cell Activation

Priyadarshini Chatterjee

Detecting infection in both a clinical setting as well as a research laboratory setting is crucial for diagnostics as well as for research purposes. One mechanism by which can be achieved, particularly in the context of viral or parasitic infected samples is through the quantification of T cell activation. Using flow cell cytometry (FACS), T cells can be fluorescently detected, however, often surface stain markers are for particular subsets of T cells such as CD4+ or CD8+ T cells rather than the population of activated T cells. This research project aims to detect the total activated T cell population.

In order to do this, we developed a fluorescently labelled recombination ICAM-1 molecule to detect increases in the molecule LFA-1, whose expression increases upon T cell activation. Hence, an increase in the molecule implied an increase in activated T cells, and this could be used as a metric of infection. This assay was optimised and established using freshly isolated PBMCs and well as in whole blood, with attempts to do so in frozen PBMCs as well. The length of stimulation with different peptide fragments was also optimised in the process. In the future, we hope to use a greater variety of peptide fragments. This was also established in healthy samples with future hopes to carry this forward into infected samples such as with patient samples or as a validation method during an infection assay set up during an experiment. We also hope to compare this with the tetramer method of staining activated T cells to compare its efficiency. If similar, this would be a much more cost-effective method of detecting infection.

Mathematical Billiards in Quadrilaterals

Hongjia Chen

Mathematical billiards is an area of study in dynamical systems, where we model billiards in an idealised environment. We stipulate that the billiard ball is a point mass that satisfies the law of reflection when interacting with the boundary. These simple constraints lead to surprisingly deep and complex dynamics. The shape of the mathematical billiard is arbitrary and we focus on quadrilateral boundaries. Polygons with interior angles of the form p/qπ, p/q ∈ Z, are well understood and can be studied using a process called unfolding. For the simplest cases, unfolding transforms the billiard trajectory to become a straight line within the tessellation of the plane. We present the necessary and sufficient conditions in order for a trajectory to be periodic in the square. Then we extend the results to the rectangular billiard and demonstrate how unfolding fails for general parallelogram billiards. We show several unexpected and interesting applications including how to solve the pouring riddle using parallelogram billiards.

Leading Risk Factors For Ischemic Stroke: A Comparative Ethnographic Study of Patients in Hawai‘i

Emily Kang

Introduction
Stroke risk factors are known to differ between ethnicities, as numerous studies have shown. However, the incidence of risk factors across underrepresented ethnicities such as Native Hawaiian or other Pacific Islanders (NHOPI) is still poorly understood, especially in different care levels such as inpatient and outpatient.

Objectives
A retrospective chart review was conducted to assess and compare the incidence of ischemic stroke and the underlying risk factors between Hawaii’s diverse ethnicities.

Methods
In this retrospective ethnographic study, data from patients diagnosed with an ischemic stroke were collected and categorized by their self-identified ethnicity. Numerous risk factors were gathered from the Hawaii Pacific Neuroscience database and split into two categories: manageable (high blood pressure, diabetes, BMI, hyperlipidemia, history of heart disease, smoking, and alcohol consumption) and unmanageable (age, gender, family history of stroke, history of TIA and history of CVA). Also, the zip code of patients’ residence was collected to know more about neighborhood average income.

Results
Age, gender, hypertension, diabetes, hyperlipidemia, Body Mass Index (BMI), and alcohol use (≥ 4 drinks/day) were statistically significant (p< 0.05) between the three ethnicities. NHOPI patients were a decade younger at the onset of stroke and were predominantly women (52.5%), whereas Asians and Caucasians were more represented by men (59.5% and 64%, respectively). NHOPI also had the highest rates of diabetes (p = 3.9x10^-5) and obesity (p = 1.26x10^-14). Although smoking and incidence of TIA were also the highest in NHPOI, data analysis rendered these results insignificant (p = 0.394 and p = 0.322, respectively). Hypertension and hyperlipidemia were higher in the Asian population, and alcohol consumption was reported more frequently among Caucasians. Analyzing the average income level of the zipcode that the patient resides in, the NHOPI group is on the lower side of the income level while Caucasians are on the higher side.

**Discussion/Conclusions**
Ischemic stroke risk factors were shown to significantly differ between underrepresented populations as observed in a similar clinical study done by Queen’s Hospital on Oahu, in which our data corroborates. Insight from specific associated risk factors through ethnicity can improve stroke prevention, care, and medical implementation to minimize stroke severity and risk.

**Future Directions**
Future studies may find a potential explanation for the varying risk factors for ethnicities, such as cultural factors, diet, environmental factors, and other social determinants of health.

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**Characterization of Isoflurane- and Ketamine-Activated Neurons in the Bed Nucleus of the Stria Terminalis**

Jiwoo Kim

The bed nucleus of the stria terminalis (BNST) is known to be associated with anxiety and regulating anxiety. The neuronal circuit within the BNST monitors environmental threat and regulates fear involving unpredictable threats (Goode et al., 2019; Somerville et al., 2010). Some anesthetics, such as ketamine, show anxiolytic and antidepressant like effects, but it is unknown whether or what kind of effect such anesthetics would have on the BNST. Recently, studies from the Wang Lab at the Duke University School of Medicine, Department of Neurobiology, have shown that anesthetics can activate certain neurons in BNST. In order to detect what types of neurons in the BNST may play a role in driving the anxiolytic effects of anesthetics, this study examined the molecular characterization of the BNST neurons activated by two different anesthetics, isoflurane and ketamine, using *in situ* hybridization process. *In situ* hybridization allows for localization of specific mRNA sequences on a whole slice by the hybridization of specific DNA probes to certain mRNA sequences and for detection by labeling the probes using fluorescence (Jensen, 2014). In this study cells expressing cFos, Penk1, and Pkc-d genes under the influence of two anesthetics, isoflurane and ketamine, were detected using *in situ* hybridization. Utilizing the immediate early gene cFos as a marker for activated neurons under the influence of isoflurane or ketamine, this study examined whether the anesthetics-activated neurons in the BNST also express enkephalin, suggested to be a peptide that reduces pain, and or protein kinase c-delta, which is responsive and activated by chronic pain (Henry et al. 2017; Wilson et al. 2019). Both anesthetics caused bilateral activation of cells expressing cFos, Penk1, and Pkc-d. For both ketamine and isoflurane activation, a portion of Pkc-d and Penk1 colocalized with cFos, thus activated due to the influence of the anesthetics. There were slight variation of colocalization between ketamine activation and isoflurane activation, but no significant difference between the two anesthetics. The findings from this study will lead to further experiments studying the roles of those cell types in the BNST and how they may cause anxiolytic effects.

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**Bending and Impact Analysis of Alternative Composite Materials Through Comparative Evaluation**

Jesse Lambrecht

There is a transition in the surfing and standup paddleboard (SUP) community to use more ecofriendly materials and move away from foam or other harmful materials. Many hobbyists and even some companies have started making surfboards and SUPs out of ecofriendly materials such as wood and hemp. Instead of using a foam interior, a skeletal structure is used to create support using spines (length of the board) and ribs (width of the board). Using a comparative approach, surfboards and SUPs made from alternative materials can be analyzed to optimize the distance between...
the spines and ribs to perform similar to a traditional board. Two main comparative factors were analyzed: the bending of the board and the impact on the board. To simulate the bending of the board, a section between the ribs is used and a moment is applied to the broken-out section. The moment is distributed between the face sheet and the core structure, this is determined by the bending rigidity of the materials. The bending section of the analysis evaluates the stress and deflection of the board in order to determine the face sheet thickness and the number of spines. The impact section breaks out a section of the board that is between the ribs and spines. A uniform pressure load is applied over the rectangular section to compare the resulting stress and deflection. The impact section is analyzed using equations for uniformly loaded rectangular plates to determine the spacing between the ribs. Using these two methods, a combination of the distance between the spines and ribs can be determined for a board to perform similar to a traditional board. The results from this research can be applied to companies working on a first-round prototype and to hobbyists creating their own board.

Development of Holistic Patient Databases to Inform Psychiatric Treatment

Clara Lemaitre

Computational psychiatry addresses the lack of foundational measures in integrative neuroscience in order to improve nosology, treatment selection, and treatment monitoring. However, for computational psychiatry research to be effective, we need large well-characterized datasets to allow accurate treatment modeling and prediction.

The purpose of the Brain Imaging-Based Care in Psychiatry study, or BIBCaP, is to longitudinally collect brain imaging and behavioral data in order to create a database that characterizes the symptoms and daily functioning of patients throughout their treatment. The data will be assessed to determine whether it has the ability to predict psychiatric outcomes. BIBCaP study will create dynamic models to inform a more scientifically grounded approach to disease identification, intervention, and prevention.

Over the course of five years, BIBCaP will administer MRIs, EEGs, and computerized cognitive tests on 1000 participants. My work is focused on adapting the computerized cognitive tests to our web application COMPAS, or the Computerized Psychiatric Assessment Suite. A HIPAA-compliant environment, COMPAS proctors and stores questionnaires and computerized cognitive tests. COMPAS houses the Dot Expectancy Test (DPX), BANDIT, WebSurf, and EEfRT tasks. Each of these evaluate symptom expression and cognitive control by measuring reward responsiveness, delay discounting, foraging behavior, and decision making.

Before recruitment was paused due to COVID-19, five participants completed the computerized assessments, three participants had MRIs, and zero had EEGs. Once data collection resumes, we will analyze the characterization of symptoms and patient functionality. We create and evaluate treatment models based on trends, correlations, variations, and outliers in the patient data. The data that passes tests for validity and reliability will be used to create robust and predictive models.

The study will produce a massive database, including holistic data about the participants, their background, their symptoms, and their treatment progression. The completed database and models will predict the trajectories of patients who exhibit similar behaviors, align with demographics, and present similarly on physiological tests. Based on these projections, clinical providers will be able to make more accurate diagnoses and informed treatment plans for their patients.

In our intent to create foundational datasets for computational psychiatry, we will need to determine to what extent COVID-19 will impair or support the validity of our data and data models. While we have added a COVID-19 based questionnaire as a part of the assessment, it will be important to separate participants based on when they started the study.

Modelling the Impact of Vaccination on the Spread of Multi-strain Dengue Virus in South East Asia

Yi Ting Loo

Dengue fever is a mosquito-borne disease that has been endemic in the tropical and subtropical areas of the world for a long time. The virus is transmitted to humans through the bites of infected female mosquitoes, primarily the Aedes aegypti mosquito. While mild dengue fever is self-limiting, severe dengue haemorrhagic fever can cause severe bleeding, a sudden drop in blood pressure and death. In week 32 2020, official data from WHO reported a total of 1,807 dengue cases in Malaysia alone, bringing the cumulative number of reported cases this year to 66,199 as of 8 August 2020.

This research derives compartmental models to investigate the dynamics of the spread of dengue fever in both humans and mosquito populations, extending the basic model...
(Keeling & Rohani, 2008) by considering multi-strain dengue viruses. Using systems of differential equations in conjunction with numerical simulations, both deterministic and stochastic models are built to explore the effects of different parameters. Using South East Asia as case study, suitable parameter values are chosen (Mordecai et al.) and sensitivity analysis is performed. Finally, the impact of vaccination is added to investigate the effect of intervention measures.

From this study, demographic factors have great influence on the dengue models with some that exhibit epidemiological oscillations. The results of this model hopefully provide an insight into the spread of dengue fever in South East Asia and also other places and contributes to suggesting optimal policies to control outbreaks, reducing the impact of the disease in the future.

Metformin Inhibits IGF-1R/mTOR/S6K1 Pathway in Pancreatic Cancer Cells Under Hyperglycaemic Condition

Septia Nurmala, Rani Sauriasari, Xian Wen Tan, Eiji Matsuura

Background
Pancreatic cancer is one of the most lethal human disease. Epidemiological studies indicated that up to 80% of pancreatic cancer patients are accompanied with either new onset type 2 diabetes or impaired glucose tolerance at the time of diagnosis. Metformin, a first line anti-diabetic drug, is emerging as a potential therapeutic agent for cancer treatment and prevention. However, the mechanisms involved in supra-physiologic condition remain unelucidated.

Objectives
This study aimed to demonstrate the potency of metformin on cell growth inhibition, FoXO1 mRNA levels and several growth factors involved in the IGF 1R/mammalian target of rapamycin (mTOR)/ribosomal protein S6 kinase I (S6K1) signalling pathway in PANC1 cells.

Methods
PANC1 cell line was used to represent pancreatic cancer. Cell viability was measured using the colorimetric assay of Cell Counting Kit-8 (CCK-8) with 0 mM, 5 mM, 10 mM, and 15 mM, 20 mM, and 25 mM glucose concentration. Cell survival rate was measured using the same assay with 0 mM, 5 mM, and 15 mM metformin concentration. All mRNA levels were measured using reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) in both normal and hyperglycaemia condition. Multiple t-test analysis was used to reveal a statistically different gene expression level (p-value < 0.05).

Results and Discussion
Metformin effectively reduced cell proliferation under conditions of normal glycaemia, and more considerably under those of hyperglycaemia. The antiproliferative actions of metformin were associated with IGF1R/mTOR/S6K1 signalling inhibition and increased FoXO1-mediated apoptosis in physiological glucose condition (5 mM). Nuclear localization of FoXO1 to exert the apoptosis effect was mediated by AMPK phosphorylation. In line with the normal glucose level, insulin/IGF-1R signalling was also inhibited in hyperglycaemia condition (15 mM glucose), although the effect is less robust. Hyperglycaemia, however, protected against metformin-induced apoptosis by AKT-mediated FoXO1 phosphorylation, a contradictory post-translational modification mechanism of FoXO1.

Conclusion
These results demonstrate that metformin has anti-tumor activities in both normal and hyperglycaemia condition involving suppression of the insulin/IGF signalling pathways. However, reduced cell growth in hyperglycaemia condition is not the direct result of the apoptosis effect of metformin. Revealing the mechanism involved in AMPK- and AKT mediated post-translational modification is needed to corroborate the finding.

Investigating D. melanogaster Cell Sheet Morphogenesis: A Live-Imaging Study of a Genomic Region Important for Dorsal closure

Daniel Tsai

Dorsal closure (DC) is a fundamental process in D. melanogaster (DM) embryogenesis and a model for cell sheet morphogenesis. DC is important because DM has genetic pathways and morphogenetic movements conserved in human development. DC occurs midway through embryogenesis and involves two sheets of lateral epidermal (LE) cells sealing a hole filled with amnioserosa cells on the dorsal side of the embryo. Although researchers have studied DC for over 40 years, the molecular mechanisms are not completely un-
nderstood. This project investigates a 118kbp region discovered during recent screens of the second chromosome that identified 62 genomic regions important for DC. When this genomic region is removed, embryos homozygous for the 118kbp genetic deficiency exhibit a severe DC phenotype involving aberrant LE cell shapes, an irregularly shaped dorsal opening, and the loss of adhesion between amnioserosa cells. Embryos in a Ubi-cadherin-GFP background that are homozygous for different sections of the 118kbp region can be obtained via a transheterozygous cross between flies of the parent deficiency (deficient for the 118 kbp region) and flies of a partially overlapping deficiency. The embryos are screened for DC defects in tissue movements using live imaging with a spinning disk confocal microscope. If the transheterozygous embryo exhibits the same phenotype as an embryo homozygous for the 118kbp deficiency, then the gene(s) responsible for the DC phenotype are within the shared deficient region of the cross. Fifteen embryos transheterozygous for the parent deficiency and an overlapping deficiency were imaged, resulting in a 54kbp homozygous deficient region. These embryos showed defects similar to the fully homozygous parent. An analysis of the defects suggests a partial phenocopy with at least one major gene responsible for the defects in LE cell shape, amnioserosa cell shape, and dorsal opening shape being located within the shared 54kbp region. Therefore, genes responsible for the reduced severity of the transheterozygous embryos, which is reflected by the lack of amnioserosa breakdown, must be located within the heterozygous deficient 64kbp region. Based on a review of genes located within the 54kbp region, the parent phenotype, particularly the LE cell defect, is a result of removing multiple genes in a pathway. Moving forward, the reintroduction of identified candidate genes including bowl, slp1, and slp2 into the fully homozygous parent will be tested using a similar breeding process. This research highlights the complexity of DC; identification of DM genes controlling DC advance our understanding of human congenital defects.

**Purification of Vanillin From Lignin Depolymerized Products**

Charles Veronee

Lignin is a complex polyphenolic organic polymer that gives vascular plants rigidity and support and represents the largest renewable source of aromatic carbon on Earth. In nature, lignin represents approximately 25% of plant cell walls, which also include cellulose (~40%) and hemicellulose (~30%). As such, in the paper making process, during the processing of wood into pulp, lignin is produced as a waste product with few popular recognized uses outside of fuel. However, lignin depolymerization has been shown to produce aromatic compounds such as phenols, benzyl alcohols, benzoic acids, and benzaldehydes. Extensive research has been undertaken to focus on the β-O-4 bond scission to produce these valuable aromatic compounds, such as vanillin (4-hydroxy-3-methoxybenzaldehyde). Recently, the conversion of commercially available vanillin into protic and aprotic benzylammonium ionic liquids was demonstrated (Socha, 2014). The resulting ionic liquids are solvents that can be used to pretreat biomass for the production of biofuels and bio-based chemicals. The primary objective of the project was to find an efficient route to create lignin-derived ionic liquids by purifying vanillin from lignin. Experiments were designed to study separation methods applied in the processes downstream of lignin depolymerization.

To maximize vanillin yield from lignin starting material via oxidative depolymerization, temperature, time, and pressure were varied statistically to achieve a maximum yield of 5.3 wt% vanillin. To further purify, extraction was coupled with column chromatography. Diaion HP-20 polystyrene resin and AG 1-X8 anion exchange resin were compared. The anion exchange resin with 1M NaCl as mobile phase purified the lignin product the most successfully with a vanillin purity of 55 wt%. Results were verified by GC-MS, HPLC, NMR, and FT-IR. Finally, the depolymerized lignin mixture was subject to reductive amination followed by acid/base extraction to isolate the aminophenol product from impurities including acetovanillone and oxidized lignin fragments in 94% purity.

This work represents the conversion of a large-volume byproduct (lignin) for the production of finer chemicals (aminophenols and benzyl ammonium ILs). In addition to biomass pretreatment, benzyl ammonium ILs are used as antiviral agents on hard non-porous surfaces. In the future, work can be done to develop additional bio-based ionic liquid materials and research their applications. Methods used here are anticipated to function with other lignocellulosic feedstocks, like hardwoods and grasses, to isolate and functionalize additional chemical feedstocks.
Effects of Cat and Dog Interactions on Urban Wildlife Admitted to a Wildlife Center in Wisconsin

Makayla Timm¹ and Nicole M. Kime¹

Small birds and mammals are often injured by dogs and cats. Some of these animals are brought to the wildlife rehabilitation centers. The number of admitted animals varies seasonally, and dogs and cats can have different effects on animals in different life stages. This study looked at 9,696 records of small birds and mammals admitted to a wildlife center in the Upper Midwest (Wisconsin) between 2014 and 2017. Data regarding taxon, species, date of admission, life stage, circumstance of rescue, and outcome were compared between dogs and cats. Data from dog and cat interactions were also compared to other causes for admission. More animals were admitted because of dog interactions than cat interactions. Dog and cat interactions are especially prevalent April through August. The proportion of birds and mammals admitted during the breeding season compared to other seasons was higher for dog and cat interactions than the same proportion for other causes of admission ($p < 0.001$). This is partially because young animals are a target. Fledgling birds were admitted more frequently than adults or hatchlings because of interactions with dogs or cats ($p < 0.001$). Mortality rate was lower for dog and cat interactions than other causes of admission ($p < 0.001$), and was lower following interactions with cats than with dogs ($p < 0.001$). Reducing the number of outdoor cats and watching free-roaming dogs more closely may reduce interactions with wildlife and decrease the need for medical assistance for wildlife because of such interactions.

INTRODUCTION

Cats are thought to pose a significant threat to the small birds and mammals upon which they prey (Loyd et al., 2013). A systematic review of the mortality of birds and mammals caused by free-roaming cats in the United States estimates that free-ranging domestic cats kill 1.3 - 4.0 billion birds and 6.3 - 22.3 billion mammals annually (Loss et al., 2013). Entire populations of birds and other wildlife species are declining or being pushed toward extinction by domestic cats (Carey, 2017). The American Bird Conservancy estimates that only 35% of cat owners always keep their cats indoors, leaving more than 30 million owned cats free to prey on urban wildlife (Burton and Dobler, 2004). Cats are primarily a threat in the early summer, during birds’ vulnerable fledgling stage (Donovan, 2012). Cats also have a high reproductive ability, having up to three litters per year yielding four to six kittens per litter (Burton and Dobler, 2004), each of which can be a threat to wildlife. There has not been much research on how domestic dogs in the United States affect wild animals. Dogs can cause physical injury, nest destruction, and death to wildlife animals (Forrest and Cassady, 2006). They can also harass or chase endemic species, which results in increased stress and energetically costly behavior among native wildlife (Lenth, 2008). A survey conducted in 2016 by the American Pet Products Association estimates that there are about 89,000,000 domestic dogs in the United States. Some domestic dogs are trained to facilitate hunting, protect property, or reduce human-wildlife conflicts by protecting livestock from people or predators (Melson, 2009).

In Wisconsin, 26 counties have wildlife rehabilitation centers or licensed rehabilitators that care for injured or orphaned urban wild animals with the intent to release them back into the wild (Wisconsin DNR). The Dane County Humane Society’s Wildlife Center (DCHS Wildlife Center) treats over 3,800 animals per year that are sick, injured, or orphaned with the goal of releasing healthy animals back into their natural habitats (Dane County Humane Society, 2020). Although data on intakes to wildlife rehabilitation centers cannot be used to estimate the total number of birds and mammals injured by domestic pets or to know how dogs and cats affect bird and mammal populations, they may provide insight into the species and life stages that are most affected. Reviewing existing data from DCHS Wildlife Center can also help other rehabilitation centers in Wisconsin understand when animals will be admitted due to dog and cat interactions, the life stages of animals admitted, and the mortality and release rates of animals.
The objective of this study was to review data regarding the impact of dog and cat interactions on urban wildlife admitted to DCHS Wildlife Center. Data from 2014 to 2017 were extracted from a commercial database (WILD-One, Wildlife Center of Virginia). Four main factors were explored. The number of small mammals and birds admitted to DCHS Wildlife Center because of a dog interaction was compared to the number of admissions following a cat interaction. The hypothesis was that more animals are brought in because of dog interactions than because of cat interactions because people are more often with their dogs when they are outside, either taking them for a walk or in their backyard. Second, the seasonal distribution of intakes due to interactions with dogs or cats was described. The hypothesis was that animals injured by a cat or dog are admitted primarily during breeding season in Wisconsin (April - August). Third, the number of admissions following dog or cat interactions was compared across life stages of birds. The hypothesis was that fledgling birds are a target for dogs and/or cats. Finally, the outcome of injuries to mammals was compared to those of birds. The hypothesis was that mortality of wildlife admitted because of cats will be greater than the mortality due to dogs because cats are actively hunting.

**METHODS**

**Animal intake and treatment procedures**

This study was based on animals that were admitted to DCHS Wildlife Center in Madison, Wisconsin between January 2014 and December 2017. DCHS Wildlife Center staff evaluates animals that the community brings in due to suspected illness, injury, or orphanage. If staff determined that an orphaned animal does not need rehabilitation (i.e., it is healthy enough to stay in the wild), they advise the community member not to step in. It is illegal for a rehabilitator to take a healthy non-orphaned wild animal into their care. Animals that are not admitted are not entered into a database.

Upon admission, licensed staff members at DCHS Wildlife Center perform physical examinations to determine the animal’s injury (e.g., physical trauma, infectious diseases, emaciation, etc.). If the same patient presents multiple injuries, the most significant injury is recorded first. Not all animals admitted to DCHS Wildlife Center are sick or injured; some are orphaned because their nest was destroyed, or parents did not come back to care for their young. The orphaned patients are still given a physical exam.

Data collected from all admitted wildlife is recorded into the database WILD-One. This includes the patient identification number (patient ID), species, date of admission, admitted life stage, circumstance of rescue, injury, disposition, and disposition date. Life stages for birds and mammals include hatchlings (birds, respectfully, that are still fully reliant on parents and/or still within the nest), fledgling (birds, respectfully, that are not fully dependent but not yet adult size), and adults (fully independent and of adult size and sexual maturity). The disposition of the wildlife animals are recorded as released, euthanized, dead, transferred, or self-released. The staff at the DCHS Wildlife Center have developed criteria for euthanasia, implemented after performing a physical exam, in accordance with the law and based on input from sponsoring veterinarians, rehabilitation experts, and federal agencies.

**Data analysis**

Data was extracted from WILD-One into Microsoft Excel 2017. Medical records from 13,454 animals, including all animals admitted for rehabilitation between January 2014 and December 2017, were initially reviewed. Reptiles and amphibians were excluded from the subsequent analysis. In addition, data from 3,758 admissions that were not small, terrestrial birds or mammals (raptors, waterfowl, specialty birds, and large mammals) were excluded from analysis. Thus, data from 9,696 small mammals and birds that were admitted for rehabilitation from 2014 through 2017 were analyzed for this study. Of these, 1,494 of the admissions were because of an interaction with a cat or dog. Cat and dog interactions were defined as a record where a wild animal was admitted to DCHS and the rescuer observed or suspected that a domestic animal and the injured wildlife animal were in contact resulting in medical care. Data on birds and mammals admitted for other causes (8,202) were used as a control. In addition to the cause of admission this study analyzed data on the species of bird or mammal, the month they were admitted, their life stage and their outcome.

To test the hypothesis that mortality due to cat interactions is greater than mortality due to dog interactions, a chi-square analysis was used to compare the proportion of each life stage the birds admitted for rehabilitation were in contact resulting in medical care. Data on birds and mammals admitted for other causes (8,202) were used as a control. In addition to the cause of admission this study analyzed data on the species of bird or mammal, the month they were admitted, their life stage and their outcome.

To investigate differences in the impacts of cat and dog interactions across life stages, only data from birds were used, as life stages are similar in bird species but not the mammals included in this study. To test the hypothesis that fledgling birds are a particular target for cats and dogs, a chi-square analysis was used to compare between seasons the proportion of breeding season intakes due to cat and dog interactions relative to the control group of animals admitted for other reasons.

To test the hypothesis that mortality due to cat interactions is greater than mortality due to dog interactions, a chi-square analysis was used to compare the proportion of animals that died following cat interactions to the proportion of animals that died following dog interactions. The null hypothesis was that the mortality rates are equal in dogs and cats. A chi-square analysis was also used to compare mortality rates following dog and cat interactions to mortality rates of animals admitted for other causes.
Table 1. Small mammal species admitted to DCHS Wildlife Center because of a dog or cat interaction. Eastern cottontails, Virginia opossums, thirteen-lined ground squirrels, common raccoons, and eastern chipmunks that came from a common nest were put together into one nest and counted as one individual. Four animals were not included in the table due to an undermined life stage.

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<th>Total Dogs</th>
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RESULTS

Dog interactions were the third leading cause of small mammal and bird admissions (9.7% of all admissions) at the DCHS Wildlife Center. Cat interactions were the eighth leading cause of mammal and bird admissions (5.6%) out of twenty-one other causes of admission.

The number of animals admitted because of cat and dog interactions varied among species (Table 1 and 2). The mammal and bird species that were most frequently admitted because of a cat interaction were the Eastern cottontail rabbit (Sylvilagus striatus, 53% of animals admitted because of cat interactions), Eastern chipmunk (Tamias striatus, 5%), American Robin (Turdus migratorius, 6%) and Mourning Dove (Zenaida macroura, 3%). The mammal and bird species that were admitted most frequently because of a dog interaction were the Eastern cottontail rabbit (72% of animals admitted because of dog interactions), Eastern gray squirrel (Sciurus carolinensis, 6%), American Robin (4.6%), and Mourning Dove (1.4%).

Overall, more mammals and birds were admitted because of dog interactions than cat interactions. Dog and cat interactions admitted more mammals than birds. More birds were admitted because of cat interactions than dog interactions, but more mammals were admitted because of dog interactions than cat interactions (Figure 1).

Mammals and birds were admitted throughout the year because of cat and dog interactions but most were admitted during their breeding seasons, between April and August (Figure 2). The proportion of animals admitted during the breeding season (compared to other seasons) was higher for dog and cat interactions for animals brought in for other reasons ($X^2 = 91.622$, df = 1, $p < 0.001$). There was no discernible difference in the number of animals admitted among the four years of the study (Fig. 2).

Fledgling birds were admitted because of dog and cat interactions more frequently than adults and hatchling birds (Figure 3). The proportion of hatchlings, fledglings, and adult admissions differed between cat and dog interactions and other causes ($X^2 = 36.568$, df = 2, $p < 0.001$).

To investigate dog and cat-related mortality rates, animals that were euthanized and animals that died either while in care or before the exam were combined into one category. Animals that were released were placed in a second category. Animals that were transferred or self-released were not included in the analysis. Animals admitted because of cat or dog interactions were less likely to die than animals
Table 2. Small bird species admitted to DCHS Wildlife Center because of a dog or cat interaction. American Goldfinches, House Finches, and American Robins that came from a common nest were put together into one nest and counted as one individual.

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<td><strong>TOTAL</strong></td>
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admitted for other causes (i.e. nest destruction, moving object, and stationary object) ($X^2 = 25.011$, $df = 1$, $p < 0.001$). When compared to all circumstances of rescue, dog interactions are the second highest reason why birds and mammals were not able to be released and cats were the fourth highest reason out of twenty-one other circumstances of rescue. The most common reason why birds and mammals do not survive is due to orphanage (16.1%), where the parents are not available, or the parents rejected their young. Dog interactions had a higher mortality rate than cat interactions ($X^2 = 34.273$, $df = 1$, $p < 0.0001$).

**DISCUSSION**

The purpose of this study was to determine if dog and cat interactions had any impact on small bird and mammal species. Four main factors were addressed. The first analysis compared the number of small mammals and birds admitted to DCHS Wildlife Center because of a dog or cat interaction. The hypothesis was that more animals are brought in because of dog interactions than because of cat interactions. The second analysis described the seasonal distribution of intakes due to interactions with dogs or cats. The hypothesis was that animals injured by a cat or dog are admitted primarily during the breeding season in Wisconsin (April - August). The third analysis compared the life stages of birds that were admitted because of a cat or dog interaction. The hypothesis was that the mortality of wildlife admitted because of cats will be greater than the mortality due to dogs.

Interactions with dogs were the third and interactions with cats were the seventh leading cause of admissions of mammals and birds to the DCHS Wildlife Center between 2014 and 2017. Dogs may have had more admissions due to people walking with their dogs and perhaps having an easier time detecting when these pets encounter wildlife or whether wildlife is injured.

Admissions for cat or dog interactions varied among species. The most common species admitted due to dog or cat interactions were Eastern cottontails, American Robins and Mourning Doves. Birds frequently forage on the ground or at feeders where they are susceptible (Mcruer et al., 2017). Eastern cottontails make nests in backyards which could lead to increased vulnerability to dogs or cats.

The effects of dogs and cats also varied among life stages. In this study, bird fledglings were found to be the most vulnerable to cat and dog interactions, perhaps because they are on the ground and unable to fly well, making them a larger target for cats and dogs. Hatchlings are less likely to be admitted than fledglings because of a cat or dog interaction; this may be because they are usually in their nest in trees. Adult birds may be caught when foraging on the ground, at a bird feeder, or due to having previous injuries (e.g. from a window strike), which can increase their chance of encountering a cat or dog. Adult birds can escape from a predator more easily and are able to defend themselves,
Unlike fledgling birds, cats are opportunistic predators and their prey selection is correlated with prey availability (Liberg, 1984; Molsher, et al., 1999).

Mammals and birds were admitted because of cat or dog interactions year-round, but fewer admissions occurred during the fall and winter months (September to March). During spring and summer months (April to August), most birds and mammals are in the breeding season, which provides domestic pets opportunities to find baby animals. In addition migratory birds are less present during winter in Wisconsin.

Many animals that are admitted into the DCHS Wildlife Center are either euthanized or die before or during care (1.35% dead on arrival). The stress of being in captivity, handling, and treatment can inadvertently increase overall wildlife mortality (Mcruer et al., 2016). It can be assumed that if the animal was not admitted and treated for their injuries the mortality rate in the wild would be greater because they are more vulnerable and may die due to their injuries.

The results in this study are significant to understanding when species are being brought in and the life stage of the animals admitted due to dog and cat interactions. Wildlife rehabilitators can use this data to understand the mortality rate, the most common species being brought into a wildlife center, and whether the species may be able to be released.

Domestic dogs and cats are one of the main reasons that small birds and mammals are being admitted into the DCHS Wildlife Center. To make sure that these animals are able to thrive in the wild and not have an interaction with a domestic pet, the number of outdoor cats should be reduced and free-roaming dogs should be watched more closely to decrease the need for medical assistance for wildlife because of these interactions.

Similar wildlife centers can use these data sets to understand how and when dogs and cats injure birds and mammals. In the future, our study could be expanded to include more wildlife centers, to compare data among regions in the United States. A larger study might compare the Upper Midwest to other national regions to determine if there are differences related to climate or culture.

**ACKNOWLEDGMENTS**

Thank you to the staff, interns, and volunteers at the DCHS Wildlife Center for treating wildlife and keeping accurate records on the wildlife admitted. Thank you especially to one of the DCHS Wildlife Center staff members, Jacqueline Sandberg for your help and support.

**REFERENCES**


The Engineering of Natural Killer Cells as an Emerging Adoptive Cancer Immunotherapy

Priyanka Senthil1* and Hariharan Balakrishnan1

Cellular therapeutics is an emerging field with significant advances in the engineering of immune effector cells, which play a revolutionary role in treatment for cancer. Although most immunomodulatory strategies focus on enhancing T cells – which have proved their ability in successful cellular therapies against leukemia – this strategy may soon face competition. Through several preclinical studies, researchers have discovered new guardian immune cells called chimeric antigen receptor (CAR)-modified natural killer (NK) cells, which show cytotoxic activity against various solid tumor types. The preclinical evidence suggests that NK cells have the same cancer-homing receptors as T cells and but need to be genetically modified to recognize and kill targets. This can be achieved by introducing CARs into NK cells and cell lines. Scientists also face the challenge of properly manufacturing engineered NK cells. If successful, CAR NK cells could be safer, cheaper, easier to produce, and more widely applicable than T cells. This review focuses on recent advances in NK cell engineering and discusses how NK cells contribute to new immunotherapeutic approaches for treatment against refractory hematological malignancies.

INTRODUCTION

In the United States, approximately 5000 people die each year from bone and soft-tissue sarcomas, which are commonly called tumors (McCarthy, 2006). Although there are new chemotherapeutic drugs, radiotherapy, and innovative surgical techniques in the clinical arsenal, there is no definite cure for malignant tumors (Arruebo et al., 2011). Therefore, in an attempt to reduce death rates, potential treatment modalities are being investigated.

One of the most talked about and promising treatments is immunotherapy. The idea of immunotherapy was first developed back in 1891 when a surgical oncologist, William Coley, investigated how the body’s immune system could be enhanced to attack malignant tumors. Coley injected more than 1000 of his cancer patients with heat-killed streptococcal organisms to cause erysipelas (a bacterial skin infection) to stimulate the body’s “resisting powers” (McCarthy, 2006). The tumors disappeared, presumably because they were attacked by the improved immune system. Seeing that the results were positive, his approach caught on. In recent years, the most prominent immunomodulatory strategy has been using chimeric antigen receptor T-cell (CAR-T) immunotherapy, which has had striking complete remission (CR) rates as high as 90% in acute lymphoblastic leukemia (ALL).

Unfortunately, CAR-T cells have some significant limitations. One of the major hardships with the generation of an autologous CAR-T cell product is that it is derived from each patient individually, making it too difficult to scale for widespread clinical use. In fact, it takes a minimum of two to three weeks to manufacture CAR-T cells (Hay and Turtle, 2017). Therefore, for a patient in critical condition with a rapidly advancing disease, treatment with CAR-T cells would be impractical. Additionally, it is difficult to collect the required quantity of lymphocytes from patients to generate CAR-T cells. And, in the case of allogenic T cells, which are transported from a donor, they can cause graft-versus-host disease (GVHD) (Liu et al., 2017).

The newly discovered CAR NK cells are perhaps more promising than the CAR-T cells. NK cells are cytotoxic, or cell-killing, and kill their targets in a non-specific manner. This means NK cells don’t have to recognize a specific antigen on viral-infected cells or cancer cells (Farag and Caligiuri, 2006; Locatelli et al., 2014). Consequently, this enhances their immunosurveillance. The NK cells decide whether to kill cells based on signals from activating and inhibitory receptors on the NK cell surface. While activating receptors ‘switch on’ the NK cell when recognizing cell-surface molecules expressed on cancer cells, inhibitory receptors ‘switch off’ the NK cell and prevent it from killing cells pos-
sessing cognate major histocompatibility complex (MHC) I molecules (Orr and Lanier, 2010). Cancer cells and infected cells become vulnerable to NK cell killing because they often lose their MHC I. Once the decision to kill is made, cytotoxic granules are released by the NK cell, leading to lysis of the target cell (Topham and Hewitt, 2009). Unlike a CAR-T cell, a CAR NK cell does not carry the risk of GVHD, and, therefore, opens the doors for development of off-the-shelf allogeneic products that could be readily available for immediate clinical use to treat thousands of patients (Yoon et al., 2010; Moretta et al., 2011). Furthermore, since CAR NK cells can retain their full array of native receptors, they have a natural ability to identify and target cancer cells. This could reduce the risk of relapse due to a loss of CAR-targeted antigen, as noted in CAR-T treatments (Sotillo et al., 2015). This would ultimately make disease escape through downregulation of the CAR target antigen less likely.

**NK BIOLOGY AND ADOPTIVE IMMUNITY**

NK cells are called “natural” killers because they have the ability to kill cancer and virus-infected cells without prior sensitization, which is crucial for cancer immunotherapy. NK cells are primarily found in the blood, liver, and spleen, but can also be found in lymph nodes (Campbell et al., 2001; De Maria et al., 2011).

As described earlier, almost all NK cell functions – degranulation, cytokine release, and cytotoxicity – are governed by signals from activating receptors and inhibitory receptors. The main activating receptors include natural cytotoxicity receptors (NCRs) and C-type lectin-like activating immunoreceptors (NKG2D), while the main inhibitory receptors include killer Ig-like receptors (KIRs) and heterodimeric C-type lectin receptors (NKG2A). Inhibitory receptors play a crucial role in ensuring that NK cells do not aberrantly activate against normal tissues, a mechanism referred to as “self-tolerance.” For example, inhibitory KIRs (iKIRs) by human leukocyte antigen (HLA) class I molecules transmit an inhibitory signal to block NK cell triggering during effector responses. However, infected cells lack HLA class I molecules (a concept called “missing self”), which means NK cells will not receive any inhibitory signal (Campbell and Hasagawa, 2013). Instead, cellular stress and DNA damage increase the regulation of “stress ligands,” activating NK receptors and signaling the NK cell to kill the target (Bradley et al., 1998; Campbell and Hasagawa, 2013).

There are various mechanisms of NK-mediated cytotoxicity. NK cells can directly kill tumor cells by releasing cytoplasmic granules containing perforin and granzyme, which prompt tumor cell lysis (Bradley et al., 1998; Screpanti et al., 2001). Alternatively, NK cells can express tumor necrosis factor (TNF) family members like FasL and TNF-related apoptosis-inducing ligand (TRAIL), which induce tumor cell apoptosis. Moreover, some NK cells contain the Fc receptor CD16 that induces degranulation against antibody-covered tumor cells, resulting in antibody-dependent cellular cytotoxicity (ADCC) (Farag and Caligiuri, 2006).

**ADOPITIVE TRANSFER OF NK CELLS TO TARGET TUMORS**

The ability of NK cells to exert rapid cytotoxicity against various hematologic malignancies such as acute myeloid leukemia (AML) (Stringaris et al., 2013), ALL (Bachanova and Miller, 2014; Roue et al., 2016), multiple myeloma (MM) (Swift et al., 2012), as well as many solid tumors including neuroblastoma, ovarian, colon, renal cell, and gastric carcinomas (Bachanova and Miller, 2014; Gras Navarro A et al., 2015) make them perfect for use in adoptive therapy. However, different tumors have developed different evasion strategies to protect themselves from NK cells. This evasion is achieved by maintaining high surface expression of HLA molecules to become invisible to NK cells (Roue et al., 2016) or by lacking ligands that signal through activating NK cell receptors. Because of this, scientists have sought strategies to enhance NK cell activity, one of which includes cytokines and artificial antigen-presenting cells (APCs) with enhanced costimulatory molecules as feeder cells for in vivo expansion. After incubation with cytokines, the NK cells gain the ability to kill tumors that are usually not sensitive to NK lysis. Scientists often combine this with monoclonal antibodies (mAb) to boost ADCC (Lin et al., 2008; Kanasawa et al., 2014; Romain et al., 2014).

In recent years, different groups of scientists have explored various methods of deriving functional NK cells for immunotherapy. Adoptive transfer of expanded, activated autologous NK cells, however, has not been very effective due to the inhibition of autologous NK cells by self-HLA molecules (Locatelli et al., 2014; Gras Navarro et al., 2015). Cells from an allogeneic source, on the other hand, have proven to be more promising for therapy. For example, as seen in preclinical studies using adoptively transferred haploidentical NK cells (NK cells from a half-matched donor used to replace damaged cells), alloreactive NK cells (cells that can recognize foreign (allogeneic) MHC molecules) can help create graft-versus-leukemia/tumor (GvL/GvT) effect while not contributing to GVHD (Ruggeri et al., 2002; Olson et al., 2010). Though the allogeneic NK cells are safe in patients with hematologic and solid tumors, they were only shown to be moderately effective in clinical activity (Yoon et al., 2010).

**CHALLENGES**

Despite the many advantages of NK cells, there is some hesitation to utilize NK cells for CAR-modified therapy due to questions about their ability to migrate to and penetrate tumor tissues. As a result, work has largely been limited to pre-clinical trials (Nayyar et al., 2019). Scientists are also rethinking the effects of the limited in vivo persistence of the NK cells because, while it increases the safety of the treatment, it may reduce its effectiveness. Although recent stud-
ies are proving to be more successful, there have been several impediments to the successful generation of CAR NK cells for clinical use. In the past, genetic engineering of NK cells, even with viral methods, reported <10% transduction efficiency (Mehta and Rezvani, 2018). The biggest challenge in CAR NK (and CAR T) cell engineering involves identifying appropriate target antigens that are pervasively expressed by tumor cells, but not expressed by normal tissue, thus limiting on-target off-tumor effects (Rezvani et al., 2017).

**STUDIES WITH CAR-MODIFIED PRIMARY NK CELLS**

There are many ways to derive functional NK cells for adoptive therapy. Expanded, activated cord blood (CB), or peripheral blood (PB)-derived NK cells have their own capabilities that play an important role in gene modification. For example, expanded, activated NK cells are known for expressing many activating receptors like CD16, NKGD2, and NCRs (Bi and Tian, 2017). NK cells have also shown to reduce tumor activity in studies with hematologic malignancies, such as AML. Furthermore, ex vivo NK cells produce a broader spectrum of cytokines including interferon (IFN)-γ, IL-3, and granulocyte macrophage colony-stimulating factor (GM-CSF), which is thought to reduce the risk of heart and kidney problems (Rezvani et al., 2017).

Most of the preclinical studies involving NK cells concentrated on targeting anti-CD19 and CD20-CARs in B cell malignancies (Imai et al., 2005; Li et al., 2010). The infusion of CD19-CAR-T cells following lymphodepletion has shown to be very positive in cases where the patient has relapsed or refractory CD19+ malignancies. However, results have not been so positive for cases where the patient has refractory Burkitt lymphoma (BL) (Rezvani et al., 2017). Scientists then began to target CD20+ aggressive B-cell non-Hodgkin lymphoma using anti-CD20 CAR mRNA-modified expanded natural killer cells in vitro and in NSG mice. The anti-CD20-4-1BB-CD3ζ CAR was then used in the gene modification of PB NK cells from a group of healthy donors. After activation with a K-562-based feeder cell line that expressed membrane-bound IL-15 and 4-1BB ligand (K562-mbIL15-41BBL), 50%–95% of the expanded PB NK cells expressed the CAR molecules. Moreover, they also displayed an enhanced in vitro cytolytic activity against rituximab-sensitive and resistant BL cells. Therefore, this also extended the survival of the Raji-xenografted mice models (Chu et al., 2015). But, in the clinical setting, these CAR molecules would likely need to be continuously infused several times due to its short-lived nature.

A recent study, however, has claimed to have found a new way to generate CAR-CD19+ NK cells that are not short-lived. The scientists genetically modified the CB-derived NK cells using a retroviral vector (iC9-CAR.19/IL15) with the gene for CAR CD19, allowing it to redirect specificity to CD19. The retroviral vector ectopically produced IL-15, a cytokine crucial for NK cell survival and proliferation, as well as expressed inducible caspase-9 (iC9), a suicide gene, that could be pharmacologically activated to eliminate transduced cells (Di Stasi et al., 2011). All these features equipped the NK cells with the genetic modifications needed to competently kill the B cell leukemia or lymphoma cells (Liu et al., 2018).

Throughout the different studies done with NK cells, various transduction strategies (most commonly using retrovirus or lentivirus-based vectors) have produced a broad spectrum of transduction efficiencies with reports ranging from 1% to 90% (Imai et al., 2005; Li et al., 2010). Lentiviral transduction is easily the most popular form of transduction because it has multiple additional benefits compared to retrovirus. For example, lentiviral transduction allows for transduction of primary, non-activated cells since it does not need actively dividing cells like retrovirus (Rezvani et al., 2017). Nonetheless, scientists are exploring other non-viral transduction methods like electroporation, which immediately expresses the CAR molecule by introducing CAR-encoding mRNA through pores. But, due to the fact that mRNA electroporation and single lentiviral transduction usually result in lower PB and umbilical CB-derived NK cell efficiencies (<10% and <30% respectively in a study), retroviral transduction may be more appropriate for gene modification of primary and CB NK cells. One way to solve this problem would be to express the CAR in induced pluripotent stem cells (iPSCs) that mature NK cells, as will be explained in the “Alternative Sources of NK Cells” section below (Hermanson and Kaufman, 2015).

**STUDIES WITH CAR-MODIFIED NK CELL LINES**

Most of the studies on NK cells have focused on the role of NK cell lines in the expression of CAR molecules, the most widely studied cell line being NK-92, which is a human cell line obtained from a patient with non-Hodgkin’s Lymphoma (NHL). The specialty of NK-92 cells is that they are missing all inhibitory KIRs except KIR2DL4 (Tom et al., 2015), allowing in vitro activity against tumor targets. In fact, NK-92 cells have been administered in over 40 patients with advanced cancer, but their efficacy is not sufficient even though they can be infused multiple times (Tom et al., 2015). This has caused scientists to turn to CAR modification in hopes to increase antitumor activity in the cells.

NK-92 cell lines, for various reasons, are theoretically thought to aid more positive results when genetically modified over primary NK cells. First, NK-92 is a well-established cell line that has been reproduced and expanded repeatedly using good manufacturing practice (GMP)-compliant cryopreserved master cell banks and is plentiful in number for cancer therapy. Due to its potential, many scientists have genetically modified NK-92 cells to express CARs like CD19 and CD20, targeting hematologic and solid malignancies for B cell leukemia and lymphoma, CD38 and CS-1 for multiple
myeloma, and HER-2 for epithelial cancers. Another specialty of NK-92 cells is that they can be given to patients through intratumoral injections, which gives them the ability to traffic to tumor sites and produce a vaccine-like mechanism effect. Additionally, due to the uniformity of the cell line, NK-92 cells are more consistent with CAR expression, and their average transduction efficiency was around 50% (Boissel et al., 2009; Boissel et al., 2013).

However, despite the fact that NK-92 cells have useful features like large-scale expansion and safety, they also have disadvantages. Some of the most important drawbacks are that NK-92 cells are potentially tumorigenic (since they have to be obtained from a patient with NHL), express multiple cytogenetic abnormalities, and have latent infections with Epstein-Barr virus (EBV) (Uphoff et al., 2010). Therefore, to ensure safety, these cells are irradiated at minimum 1000 cGy before clinical use, though this reduces their in vivo proliferation, persistence, and long-term antitumor efficacy (Uphoff et al., 2010). Moreover, though NK-92 cells have the ability to be repeatedly infused, continuous infusion may result in rapid rejection and cellular immunity against the allogeneic cell line.

ALTERNATIVE SOURCES OF NK CELLS

Another source where NK cells usable for CAR expression can be extracted is from human pluripotent stem cells (HPSCs), since both human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) produce a limitless number of NK cells. Lowe et al. developed a strategy for the differentiation of NK cells from CD34+ human HPSCs isolated from cryopreserved CB, which were then modified to express CD19-CAR. They also described a platform to express other CAR molecules by using a feeder-free protocol for the generation of gene-modified NK cells from HPSCs using insulin-like growth factor 1 (Lowe et al., 2016).

CARs TARGETING ACTIVATING RECEPTORS OR OTHER NK CELL SIGNALING MOLECULES

The CAR-NK constructs that have been explained above all deal with the intracellular signaling chain CD3ζ, conferring specific cytotoxicity to surface-tumor antigens. An alternative strategy is developing CAR-NK cells that target ligands for activating NK receptors like NKG2D. The NKG2D ligands, major histocompatibility complex (MHC) class I chain-related A (MICA), MICB, and several UL-16-binding proteins (ULBPs) cover tumor and virally infected cells. This is the reason why an NKG2D CAR can identify almost all (90%) human tumor types and on immunosuppressive cells expressing NKG2D ligands, such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) (Chang et al., 2013). However, the ligands bring up the challenge of “on-target/off-tumor” toxicity, as they are produced during many physiological circumstances, such as inflammation. NKG2D CAR cells prompt enhanced cytotoxic activity because NKG2D cells lack signaling motifs, and the NKG2D CAR, when ligated, sends a signal via the phosphorylation of DNA-activated protein 10 (DAP10), which in turn recruits downstream signaling effector molecules (Chang et al., 2013). In this study, the researchers wanted to see if supraphysiologic activating signals could enhance NK-mediated cytotoxicity, and co-expressed DAP10 with the NKG2D/CD3ζ CAR. They tested the activity of NK cells transduced with this CAR against multiple cell lines from various malignancies. Intriguingly, there were positive responses with all the cell lines, including osteosarcoma, prostate carcinoma, and rhabdomyosarcoma. However, this strategy resulted in the loss of activating ligands on few primary hematologic malignancies, ultimately affecting NKG2D-mediated cytotoxicity. The authors, surprisingly, did not find any correlation between the level of NKG2D ligand expression and NKG2D-DAP10-CD3ζ receptor-mediated cytotoxicity (Chang et al., 2013).

In another study, Topfer et al. wanted to identify a CAR that could activate NK cells using a different method, and incorporated DNA-activation protein 12 (DAP12) and prostate stem cell antigen (PSCA) scFv (derived from the hybridoma 7F5) in primary NK cells and the NK cell line YTS (Topfer et al., 2015). While DAP12 is expressed in NK cells (as is in many activating receptors), the anti-PSCA-DAP12 CAR is expressed in primary NK cells as well as the YTS-NK cell line, and has the ability to lyse otherwise resistant PSCA+ HLA-B/C- and HLA-C-matched tumor cells. Interestingly though, the anti-PSCA-CD3ζ-based CAR did not enhance cytotoxicity as well as the YTS-NK CAR incorporating the DAP-12 signaling domain. This finding was crucial. To this date, it is the first to show that a single immunoreceptor tyrosine-based activation motif (ITAM)-containing DAP12-CAR has the ability to signal as effectively as a CD3ζ-based CAR containing three ITAMs. Additionally, this DAP12-signaling CAR did not need any extra costimulatory signaling molecules for in vitro activation and cytotoxicity (Topfer et al., 2015).

While CAR modification is an effective immunotherapy, engineered NK cells can also increase cell cytotoxicity by expressing cytokines. This strategy might be even more beneficial than CAR modification, as it increases NK cell persistence while also eliminating the need for a toxic in vivo cytokine supplementation. A major challenge is overcoming the risk of inducing CRS, cytokine-induced systemic toxicity, and malignant transformation in the transduced cells. Some scientists proposed the idea of temporarily introducing genes coding for IL-2 or IL-15 using short-lived expression models like mRNA electroporation to avoid toxicities.

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SPECIAL CONSIDERATIONS FOR CLINICAL TRANSLATION OF CAR-NK CELL THERAPY

There have been clear positive results with both CAR-NK cells and a strong safety profile for non-genetically modified NK cells. However, there have only been two clinical trials of CAR-NK cell therapy with patients: NCT00995137 from St. Jude Children’s Research Hospital and NCT01974479 from The National University Health System, Singapore. The two trials are using an identical second-generation anti-CD19 CAR with the 4-1BB costimulatory domain (anti-CD19-BB-ζ) to target refractory CD19+ ALL (Shimasaki et al., 2012). Though the Singapore trial is enrolling both children and adults, the St. Jude trial was only open to pediatric patients and is no longer accepting patients. This dose-escalation trial gives patients a single intravenous (i.v.) infusion of anti-CD19-BB-ζ NK cells at doses of 0.5 × 107 to 1 × 108 CD56+ cells/kg, and the clinical results are still being awaited.

In the last year, many more studies regarding CAR-NK cell therapy were conducted and registered on ClinicalTrials.gov. One example is PersonGen BioTherapeutics, which was given permission to administer sequential doses of third-generation (relevant scFv attached to TCRζ, CD28, and 4-1BB signaling domains) CAR-transduced NK-92 cells (on days 0, 3, and 5). The scientists in that group are focusing on targeting refractory CD7+ leukemia and lymphoma in adults (NCT02742727), CD33+ myeloid malignancies in children and adults (NCT02944162), refractory CD19+ ALL malignancies in patients undergoing hematopoietic stem cell transplantation (HSCT) (NCT02892695), and MUC1+ relapsed and refractory solid tumors (NCT02839954). Other scientists are working to determine the safety and efficacy levels of escalating doses of off-the-shelf CB-derived NK cells, which express iC9.CAR19.CD28-ζ-2A-IL-15 for relapsed or refractory B-lymphoid malignancies.

Undoubtedly, there are several challenges and questions that must be answered before CAR-NK therapy can be used to treat a larger number of patients. Although many trials have explained strategies for isolation, expansion, and transduction of NK cells, the generation of the cells is still lengthy and difficult. While retroviral constructs have greater efficacy than lentiviral constructs, they have a greater risk of contributing to the development of insertional mutagenesis, creating a regulatory hurdle. In order to avoid the risks of oncogene activation and insertional mutagenesis, several scientists have used electroporation. Unfortunately, the studies have reported a very low success rate, with transfection efficiencies as low as 10% (Boissel et al., 2009). Additionally, since CAR molecule expression usually lasts less than 7 days, it is likely to lower the long-term efficacy level of the CAR-NK cells (Zhao et al., 2010).

Another question that still needs to be studied is whether the infused allogeneic CAR-NK cells (CAR-NK cells from a donor, rather than from self) will be rejected, and if so, whether lymphodepletion will be necessary. The problem is that lymphodepleting chemotherapy will deplete other immunosuppressive cells within the tumor-like Tregs and MD-SCs. This will hurt NK cell cytotoxicity and in vivo expansion. Moreover, due to some of the safety questions raised with infused CAR-modified T cells, scientists are looking into whether a suicide system (e.g., based on caspase-9 or thymidine kinase), or programmed cell death, will need to be incorporated (Zhao et al., 2010; Di Stasi et al., 2011; Zhou et al., 2015).

Despite the several preclinical trials done following CAR-NK and CAR-T cells, there are still many unanswered questions. These include whether repeated infusions could trigger immunogenicity, elicit human anti-mouse antibodies (HAMAs), or cause cellular-mediated rejection/sensitization in CAR-NK cells. The question of HAMA immunogenicity, however, has been explored in CAR-T cells, and has proven not to be a concern, likely due to the fact that many of the patients have received a single infusion of autologous cells.

Without doubt, in order to establish a safe and effective CAR-NK immunotherapy, further studies regarding the optimal vector, construct, and transduction method are needed.

CONCLUSION

We are in an exciting era in the field of cellular therapy, where many new ideas for cancer treatment are being explored. The theory of NK cell immunotherapy is one of the most promising strategies against refractory malignancies due to its high cytotoxicity. NK cells have proven to be significantly more diverse than once believed and have shown great potential in tumor control and immunosurveillance. Most importantly, NK cells hold great promise for the development of an off-the-shelf cellular product that could eliminate the need for a patient-specific diagnosis, making them readily available for immediate clinical use. Although much has been discovered, there are still numerous scientific questions and regulatory hurdles that must be addressed before NK cells can be extended to larger cohorts of patients. For example, it is very important to identify the ideal vector, signaling endodomain, and costimulatory molecule for NK cells that provides the best response and safety profile. In order to do this, different combinatorial techniques will have to be tested to improve the efficacy of tumor-specific NK cells. This will likely be done by harnessing the innate power of the NK cell, inhibiting or knocking out immune checkpoints, or by targeting the tumor microenvironment. Furthermore, additional gene editing techniques like CRISPR/Cas9 will have to be analyzed in the setting of NK cells. Targeted genome editing has shown to be more effective with CRISPR-Cas9 ribonucleoprotein (RNP) complexes using a nuclear localized signal (NLS)-tagged Cas9 when compared to Cas9 plasmid transfection. CRISPR-Cas9 RNPs substitute the need for plasmid transcription and translation, circumvent NK cell...
sensitivity to DNA, and increase nuclear delivery (Riggin et al., 2020). Currently, only one clinical trial recruiting patients is exploring this strategy, although trials targeting immune checkpoint inhibitors like anti-PD-1 could help control malignant melanoma (EbioMedicine, 2019). Without doubt, therapeutic strategies designed to leverage engineered NK cells will make a significant contribution to the recent paradigm shift in cancer treatment.

REFERENCES


A Novel Nickel-Titanium Wire-Actuated Prosthetic Motor Clutch

Andrew Chan1*, Jacob Altholz2, Richard Weir2, Matthew Davidson2

The standard motors that drive prosthetic fingers cannot provide both the speed and torque required to hold objects as efficiently as a human hand. This problem of high speed/torque can be solved by using multiple motors or transmissions to drive a prosthetic finger, but these increase weight, cost, and complexity of the prosthetic finger system, which lead to people abandoning their device. Presented here is a novel clutch mechanism that alleviates the high speed/torque problem by holding the motor in place during gripping using nickel-titanium “memory wire” called Flexinol. This clutch mechanism allows an inexpensive low-torque motor to drive fingers while retaining the grip strength benefits of a high torque motor thus reducing cost and weight of the prosthetic finger system. The newly developed clutch presented in this paper was compared to our earlier nitinol clutch design (described in Altholz et al., 2015) and to a clutchless motor, which served as the control. The direct effect on torque resisted using one, two and three strands of Flexinol within the new clutch design was measured in addition to the torque resisted by the alternate clutch designs. The maximum torque each clutch could withstand before failure (forced motion) was found by applying a torque to the motor with a weighted bar. The clutch design presented in this paper resisted significantly more torque than the older clutch and the control system (p < 0.001). Increasing the number of nitinol strands also increased the torque the clutch could provide. Clutch 2 with three strands of nitinol had a failure torque of 0.93 with a standard deviation of ± 0.21 Nm. This is a torque that is significant enough to withstand forces encountered in by prosthetic users and is within a margin of error of the industry standard minimum torque of 1 Nm. Therefore, our novel clutch can resolve the high-speed/high torque problem while reducing cost and weight.

INTRODUCTION

The earliest known prosthetic hands originated in ancient Egypt (Zuo and Olson, 2014). These early designs were mostly cosmetic representations of hands and provided little functionality (Zuo and Olson, 2014). Systems using cables as actuators emerged in the late 1700s and became commercially available in the mid-1800s (Zuo and Olson, 2014). These prostheses allowed the user to open or close a gripping hand by pulling a cable with the opposite arm. Cable-driven systems are frequently used due to their low cost and mechanical simplicity, but the repetitive motions required to operate them can lead to over-use shoulder injuries (Weir, 2004). The first motor-controlled devices were invented in the early 20th century and have seen dramatic improvements in recent years. The most advanced of these devices, myoelectric prosthetics, can respond to signals from muscles in the remaining limb to control anatomically similar fingers to grip many different objects (Geethanjali, 2016). However, as dexterity and strength increase, so do weight, complexity, and cost.

An intact hand picks up an object in two phases: a high-speed, low-torque reach phase where the hand is moved to the object, and a low-speed, high-torque grasp phase where the hand conforms to the object and applies appropriate force (Weir, 2004). Prosthetic devices, however, must strike a balance between efficiency and functionality (Weir, 2004). Motors in a prosthetic hand can recreate this grasping process using a low-torque/high-speed motor during the reach phase and high-torque/low-speed motor during the grasp phase (Weir, 2004). However, this requires an engineering choice as a single system cannot satisfy both of these torque/speed constraints: 1) including both motors which is effective but increases weight and cost 2) using only a high-torque motor which allows for grasping and lifting of heavier objects but is slow and draws more power, and 3) using low-torque motors that are inexpensive and quick, but can be easily driven in the opposite direction even when engaged, making it difficult to hold heavy objects. Typically, the industry standard is to sacrifice weight for effectiveness which can often lead to device abandonment (Weir, 2004). The negative consequence
of the third design choice is known as “back-driving” and is a major issue for prosthetic devices. Optimizing this choice to limit the ability of motors in a prosthetic hand to be back-driven is essential for designing effective prosthetic devices.

The solution presented here is a mechanism that grasps the motor drive shaft like a clutch to prevent back-driving when subjected to forces that someone would encounter in every-day life. While purely mechanical clutches exist, they are complex, heavy, and expensive, and usually do not allow the user to control when they activate. Therefore, this clutch uses nitinol “memory wire” which contracts to actuate a simple caliper system and could be controlled by the prosthesis’s existing electronics. Including a clutch overcomes the major limitation of high-speed/low-torque motors: their back-drivability. The back-drivability refers to the extent to which the motor can be driven in the reverse direction. It also reduces weight and complexity and only costs about $10 to manufacture. To our knowledge, it has not been previously used in the specific application we describe in this paper.

Presented here is analysis of two nitinol-activated clutch designs using different numbers of nitinol strands. This new clutch design prevents back-driving better than both an unclutched motor and the clutch described by Altholz et al. (2015). Altholtz et al. introduced a basic nitinol-actuated clutch design with demonstrated minimal functionality. This paper expands on their results by demonstrating that a low-torque motor can have the low back-drivability benefits of a high-torque motor when combined with the new clutch. Integrated into prosthetic hands, this new design could reduce cost and weight in commercial prosthetics. The new clutch was tested with one, two, and three nitinol strands. The authors of this study hypothesized that increasing the number of strands would increase the amount of torque the clutch could resist. Since the force of friction is linearly correlated with the applied normal force ($F_f = \mu F_N$), and each strand applies force independently, the resistive increase from adding more strands was expected to also be linear.

**METHODS**

The clutches presented here work by using “nitinol memory wire”, an alloy of nickel and titanium that can be bent and twisted holding its shape like a normal wire (e.g. copper or aluminum), but that returns to its original shape when electricity is applied to it (Khan, Muhyuddin, and Wadood, 2017). This clutch uses a proprietary brand of nitinol, called Flexinol, that contracts when subjected to a low-voltage current instead of simply returning to a user-set shape (DYNALLOY, n.d.-a).

The Flexinol was wrapped around two semi-circular metal calipers around the motor shaft so that, when current was applied to the wire, the calipers contracted and applied force to the motor shaft to hold it in place (Figures 1 and 2a). This allows a prosthetic hand to maintain a grasp on heavy objects even when using a low-torque motor. Flexinol
was chosen for this study because it is the industry standard for contractile wires, and the only brand for which technical specifications are available. In addition, using commercially available components when available reduces cost ($4.50/meter (DYNALLOY, n.d.-b) compared to a custom manufacture process), improves test repeatability, and scales for mass production. Designing and manufacturing novel memory wire was beyond the scope of this project and the capabilities of this lab.

Design and fabrication
The clutch calipers were designed with Solidworks CAD software (Dassault Systemes, Vélizy-Villacoublay, FR) and 3D printed out of tool grade, high nickel content steel using an EOS M270 printer (EOS, Krailling, GR). They were fabricated on a CNC mill at the University of Colorado at Denver machine shop to remove support material. The calipers were attached to the motor housing by a screw and fit around a plastic sheath on the drive shaft (Figures 1 and 2). A polyethylene plastic was chosen for the sheath to minimize cost and improve durability. Since the collar was not in contact with the nitinol, there was no concern that any heat from the wires would damage it. However, a commercial design should consider an even more durable material. All tests were performed with a Faulhaber MM1724 DC motor (Faulhaber, Petersburg, FL) the collar of which had been modified to accommodate the clutch. A 0.4 mm Flexinol wire (DYNALLOY, Irvine, CA) was threaded through the calipers and out through a hole in the motor collar. This wire was secured to two bolts in the test support (Figure 3). Current was provided by a power supply (BK Precision 1666) set to provide 5 V and limited to 1000 mA. Current passed through the Flexinol caused it to contract the calipers around the drive shaft. The test configurations are shown in Figure 4 and described in Table 1.

Configuration A served as a control to test the system with no clutch. Configuration B tested the clutch described in Altholz et al. (2015), which had non-concentric calipers and used one strand of Flexinol. The configurations of C used Clutch 2 which included concentric calipers, a plastic collar around the motor shaft, and a washer between the clutch and the gear box to prevent jamming (Table 1). Configuration C was tested with one, two, and three strands of Flexinol. At three strands, current draw exceeded 1000 mA and overheating became a concern, so no additional strands were tested.

There is no formally established benchmark for motor torque in the literature. Although some prosthetic hands produce as much as 2.5 Nm torque at the metacarpophalangeal joint (knuckles), 1 Nm was chosen as the minimum torque criterion because it is the industry standard used for the German-developed DLR/HIT Hand II (1.05 Nm) and the prosthetic devices from Liu et al. (2008).
Experimental techniques
This test was intended to mirror forces that a prosthetic user would need to exert during activities of daily living. To measure torque at failure, the clutch and motor were secured to the lab bench with rubber-padded metal clamps and a 0.28 m long hardened steel bar weighing 105.28 g was attached to the motor shaft with a set screw 2 cm from its end (Figure 5). Hardened steel was used to eliminate any bending effects from the measurements. The strength of the motor-clutch assembly was measured by attaching the bar to the output shaft and rotating it until it fell, at which point the angle was recorded. Angles were measured by hand with a goniometer. Each trial was performed 10 times.

Torque at failure was calculated from the mass and angle of the bar by

\[ \tau = \frac{l}{2} F \sin(\theta) \]  

(1)

where \( l \) is the length of the bar from the pivot point (0.25 m) and \( F \) is the mass of the bar multiplied by \( g \), acceleration due to gravity, and \( \theta \) is the angle at failure.

The new clutch overcame the maximum torque the bar could provide alone. Therefore, to find the maximum torque, a cup was attached 0.24 m from the pivot point and lead shot was slowly added in approximately 0.5 g increments until the clutch failed and the bar fell. The total mass of both the cup and the added load was recorded at failure. The bar was set in position before the clutch was engaged. Torque was calculated according to the following equations

\[ \tau_{\text{bar}} = \frac{l_{\text{bar}}}{2} \times F_1 \]  

(2)

\[ \tau_{\text{cup}} = l_{\text{cup}} \times F_2 \]  

(3)

\[ \tau_{\text{net}} = \tau_{\text{bar}} + \tau_{\text{cup}} \]  

(4)

where \( l_{\text{bar}} \) is the length of the bar (0.25 m), \( l_{\text{cup}} \) is the distance of the cup from the pivot point (0.24 m) and \( F_1 \) is the mass of the bar multiplied by the acceleration due to gravity \( (g) \) divided by two. \( F_2 \) is the mass of the cup and added mass multiplied by the acceleration due to gravity \( (g) \) divided by two.

Table 1. A comparison of the different clutch designs with their corresponding figures.

<table>
<thead>
<tr>
<th>Configuration name</th>
<th>Gear box</th>
<th>Clutch name</th>
<th>Caliper characteristics</th>
<th># strands</th>
<th>Collar?</th>
<th>Washer?</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Control</td>
<td>415:1</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>B: Design 1</td>
<td>415:1</td>
<td>Alholtz Clutch</td>
<td>Wide, non-concentric</td>
<td>1</td>
<td>none</td>
<td>none</td>
<td>4a</td>
</tr>
<tr>
<td>C1: Design 2, 1 strand</td>
<td>415:1</td>
<td>Design 2</td>
<td>thin, concentric with motor shaft</td>
<td>1</td>
<td>plastic</td>
<td>between clutch and gearbox</td>
<td>4b</td>
</tr>
<tr>
<td>C2: Design 2, 2 strands</td>
<td>415:1</td>
<td>Design 2</td>
<td>thin, concentric with motor shaft</td>
<td>2</td>
<td>plastic</td>
<td>between clutch and gearbox</td>
<td>4b</td>
</tr>
<tr>
<td>C3: Design 2, 3 strands</td>
<td>415:1</td>
<td>Design 2</td>
<td>thin, concentric with motor shaft</td>
<td>3</td>
<td>plastic</td>
<td>between clutch and gearbox</td>
<td>4b</td>
</tr>
</tbody>
</table>

Figure 5. A simplified drawing of the testing setup depicted in Figure 3.

Figure 6. Comparison of the torque of the different systems and statistically significant differences which are indicated by the stars. There was no significant difference between the torque for the unclutched system and design 1, nor was there between design 2 2-strand and design 2 3-strand. All other combinations experienced significant differences.
The maximum failure torque of each configuration and clutch design is shown in Figure 6. Important key results are described below.

A one-way ANOVA indicated that Clutch 1 did not significantly increase maximum torque compared to the unclutched system. The failure torque of Clutch 2 was higher than both the unclutched system and Clutch 1 (Tukey HSD). Within Clutch 2, one-strand and two-strand configurations performed similarly (better than Clutch 1 and the unclutched system), as did two and three strand configurations. The three-strand configuration had a significantly higher failure torque than the one-strand configuration (Tukey HSD). Error was calculated using the ANOVA test.

A linear relationship was found between torque and number of strands of Flexinol ($R^2 = 0.9964$).

### DISCUSSION

Clutch 1 did not perform as well as anticipated. The average 0.079 Nm of torque was far from the desired 1 Nm and there was no significant difference between the Altholz clutch and the unclutched system. The key deficiency of this design was that the calipers were not concentric with the drive shaft, resulting in a single point of contact and reducing the applied friction. This demonstrates that Clutch 1 would not be effective in a real-world scenario as it was not a significant improvement over the unclutched motor. Additionally, Clutch 1 was prone to jamming due to its large caliper size.

Clutch 2 was a significant improvement over Clutch 1 and the unclutched system (Figure 3). The greatest failure torque was found in the two and three nitinol strand configurations of the new clutch. Creating concentric calipers and adding a plastic collar to the drive shaft allowed the system to resist 0.93 Nm ± 0.21 Nm of torque at failure with three strands of Flexinol. This meets the requirement for resisting real-world torques and appears to be the maximum torque achievable with the current design.

### The Effect of Increasing Strands

Increasing the number of strands of nitinol linearly increased the maximum torque by 0.16 Nm per strand ($R^2 = 0.996$). However, two factors limit further increasing the number of strands. First, increasing strands also increased the current draw. While current draw was not measured during the testing (it was regulated to 1000 mA), it was noted that the unregulated current draw with three strands was over the power supply limit of 1000 mA. This heated the wires to the point of becoming incandescent. In contrast, one strand drew only about 200 mA. With more wires, there is concern that the nitinol would reach the temperature at which it could melt or interfere with other components of the prosthetic system.

Second, increasing the number of strands noticeably increased the time it took for the wires to cool enough to relax the clutch system. This time measurement was not part of the experimental design but was estimated to be shorter than one second with the other configurations and about four seconds with three strands. This is likely too long to be useful in a prosthesis since people need to be able to release objects quickly.

### Conclusions

This study has shown the simplicity and effectiveness of a nitinol clutch system to prevent back-driving in motors for prosthetic hands. The three-strand Clutch 2 resisted 0.93 ± 0.21 Nm of torque, which achieved the goal of 1 Nm. The calipers were easy to manufacture and implement, and the cost of materials was very low (approximately $10 per unit). The rest of the system was comprised of parts that are commercially available. As such, this clutch would be a viable option for large-scale production and use.

This study shows for the first time that nitinol wire clutch systems could have a promising future in prosthetics. Building prosthetic fingers that can be driven using inexpensive low-torque motors while still being able to grasp heavy objects will improve functionality and reduce abandonment rates.

Two additional targets for improving this clutch design are: the motor shaft coating and the Flexinol wire thickness. In this design, the motor shaft collar was made of SLA cured resin plastic. Although this coating survived the testing without incident, a collar made of a more durable material, such as a metal, could increase the friction and therefore the amount of torque resisted. Second, increasing the thickness of the wire could increase torque by providing a greater applied force to the central drive shaft. This additionally could resolve the current draw and reaction time limitations.

<table>
<thead>
<tr>
<th>Configuration Name</th>
<th>Torque at Failure</th>
<th>Deviation +/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Control</td>
<td>0.030032</td>
<td>0.005014</td>
</tr>
<tr>
<td>B: Design 1</td>
<td>0.065</td>
<td>0.027</td>
</tr>
<tr>
<td>C1: Design 2, 1 strand</td>
<td>0.61</td>
<td>0.16</td>
</tr>
<tr>
<td>C2: Design 2, 2 strands</td>
<td>0.78</td>
<td>0.16</td>
</tr>
<tr>
<td>C3: Design 2, 3 strands</td>
<td>0.93</td>
<td>0.21</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS
Authors would like to thank Jac Corless and Stephen Huddle for their assistance in the manufacture and machining of the clutch calipers and housing.

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Misinformation in a Global Pandemic: Where Does it Come From and How do we Stop it?

Habiba Abbasi

The streets of London grow empty as businesses, universities, schools, stores and train stations are instructed to close. One would think it’s a public holiday, Christmas day perhaps. Everyone is at home spending time with their loved ones, taking a break before the New Year comes around with new goals and ambitions to achieve. But wait, that was just the end of March.

The coronavirus pandemic has taken the world by surprise, causing mass disruption to education, the economy, our emotional and social lives. With a considerable amount of time to spend idly and a lack of understanding about what is actually going on around the world, how did coronavirus start? Why now? What can we do to protect ourselves? It’s only reasonable to turn to the internet for answers.

The internet contains a vast array of resources and information, however, it is also home to the spread of misinformation as people tend to forward information without fact checking. In fact, a shocking 59 percent of shared URLs on Twitter are never opened by the user sharing the content according to a paper published by the Association for Computing Machinery in 2016.

CONSPIRACY THEORIES

Scientific organisations have long acknowledged the role of misinformation and conspiracy news in turning the public away from science. In a paper published in the Proceedings of the National Academy of Sciences, researchers describe a key difference between scientific and conspiracy news: the ability to verify the content being published.

Conspiracy theories tend to reduce the complexity of reality by explaining events as plots conceived by powerful individuals or organisations, fuelling distrust in mainstream society and from official recommended practices.

Accepting theories that cohere with their belief system leads individuals to believe in false information that is presented to them online. A paper published in the Public Library of Science explains that the danger of accepting false beliefs is that they are rarely corrected once adopted by an individual. What’s more is that online platforms use this cognitive bias to their advantage, making individuals more susceptible to misinformation, according to an article published by Nature in May 2020.

This isn’t the first time the world has dealt with the spread of misinformation, conspiracy theories and false information have always existed. Rory Smith, Research Manager at First Misinformation in a Global Pandemic: Where Does it Come From and How do we Stop it?

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THE ROLE OF SCIENTISTS IN THE SPREAD OF MISINFORMATION

In correspondence with Dr Emily Dawson, Professor in the Department of Science and Technology Studies at UCL and author of Equity, Exclusion and Everyday Science Learning we were able to learn more about the role scientists play in the spread of misinformation on the internet.

“I don’t think the scientific community can ensure people receive evidence-based information. This is simply beyond their power”, Dawson weighs in.

However, data on public attitudes to science encouragingly shows that public confidence in science and scientists is actually very high according to Dawson.

“Working with the public rather than at them would be a really big help in terms of building support, sharing information and staying relevant”, Dawson said.

Dawson’s book Equity, Exclusion & Everyday Science Learning explores how some people are excluded from science education and communication, and the book also develops a framework to support inclusive change.

“We know from research that people don’t make decisions based on information, they make decisions based on how they feel about the information, how they feel about the person/place where they found the information”, Dawson says.

Dawson’s book explores the experience of ethnic minorities in everyday science learning, exploring a theory of exclusion and suggesting a theory of inclusion.

“Those involved in science communication need to carefully design their media and communication strategies by creating clear and accessible information sources and work to be in spaces that people get their information, even if that’s Instagram!” she adds.

We also need to develop a better understanding of how people make sense of information, how communication works and what might make ‘false information’ appealing and interesting according to Dawson.

PEER REVIEW

Peer review is a vital component in the dissemination of scientific research. No claim can be considered valid until it has been peer reviewed. During a time of crisis this process has proven to be ineffective because decisions on the validity of results need to be made much more quickly than is possible under the current peer review process.

To tackle this problem, preprint servers such as medRxiv have been established to allow the rapid dissemination of research that have not been certified by peer review. However, information on these servers may or may not be reliable, raising doubts for example about governmental advice on the mandatory use face masks in public.

The guidelines on the use of face masks have shifted throughout the pandemic, with the WHO first advising not to use face masks to now making them mandatory following publications of evidence showing that masks can prevent the spread of Covid-19. Nevertheless, this only fuels the confusion and doubt among the public.

MOVING FORWARD

Although algorithms are highly beneficial to providing a personalised user experience on the internet and maximising user engagement, scientists have suggested that algorithmic solutions are not useful in the propagation of misinformation on the internet. Algorithms track user clicks and provide content consistent with the individuals preferences. Scientists suggest that algorithms should maintain more diversity and not entirely cater to what the user is usually engaged with to present a more balanced version of reality in cases such as during the coronavirus pandemic, and this could ultimately offer one approach of combatting the spread of misinformation online.

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A crucial step in a scientific manuscript’s publication, scientific editors find themselves at the intersection of the science and business world. They remain in touch with the innovative culture of science, while staying away from the lab bench, making this job sector a perfect environment for people with a keen interest in science, but who want to leave the white coat behind.

Scientific research papers are the primary way in which scientists communicate their findings to the wider community. However, a critical process of reviewing and editing occurs before these papers are finally published in scientific journals. So, scientific editors work closely with the paper’s authors to improve their scientific manuscripts, with the final goal of producing a final product that clearly portrays the scientific findings to the readers.

Recently, I had the pleasure of interviewing Lesley Anson, who has established herself in the world of science editing and communication. By working as a scientific editor at Nature, managing her own established journal and more recently starting up her own freelance consulting company Anson Scientific, Lesley has been able to develop critical analytical skills and see this sector from every possible angle.

Before she had even aspired to enter the world of scientific editing, Lesley had learned how to critically review scientific papers in her undergraduate degree studying Physiological Sciences at Newcastle University. During her undergraduate degree, she spent an entire module on reviewing and editing scientific manuscripts, which was invaluable to her career.

‘I didn’t know then, but I was actually developing a key skill, which I had to use every day when working at Nature,’ Lesley said. ‘I actually think this was one of the main reasons I landed my first job as a scientific editor.’

After her undergraduate degree, Lesley started a PhD in Auditory Biophysics at the University of Bristol. Throughout her 3-year PhD, she researched the mechanisms of how our brains recognise and translate sound. She did this by focusing on which chemical neurotransmitter signals transfer auditory information from the first point of contact, the cochlear inner hair cells, into the rest of our brains. Lesley’s early work, published in the Journal of Physiology in 1995, supported the hypothesis that the excitatory neurotransmitter glutamate was essential for the first steps of this transduction pathway. Characterising these complex signaling pathways is crucial for the development of hearing loss therapeutics, as hearing loss is often caused by overstimulation of these auditory receptors (Raphael 2002).

From her PhD, Lesley found that she had a keen interest in neurobiology and glutamate signal transduction. She continued her research within this narrow avenue, by taking a postdoctoral position at University College London, researching the ionic glutamate receptors: N-methyl-D-aspartate (NMDA) receptors, publishing these findings in the Journal of Physiology in 1998.

She fully enjoyed this experience, as she enjoyed the fast-paced working environment of lab work and getting to consistently learn new techniques. Yet, she knew that in the long term she did not want to pursue a career in academia.

‘When I was looking to change the direction of my career, by chance I saw an advert for a position at Nature as an editor of their molecular neurobiology manuscripts,’ Lesley said. ‘I applied and got the job! I would definitely say I fell into this new working chapter of my life, but it allowed me to continue being close to the parts of science that I loved. Learning about new interesting scientific findings and still being interconnected with the scientific community, without being in the lab.’

Photo curtesy of Lesley Anson.
Lesley explained how when she approached a new paper she had to keep in mind two major questions. The first looked at: ‘Is this paper suitable for the journal?’ Nature for example was looking for big, surprising findings, which would apply to multiple scientific fields. She then also had to make a technical decision, looking at both the experimental design and the solidness of the conclusions concerning the presented results. This process involves a highly stringent, peer-reviewed process, which includes getting opinions from other experts in the field.

‘When I was a Scientific Editor, the job was highly interconnected and diverse,’ Lesley said. ‘I had to establish strong relationships with my colleagues at Nature, the paper’s authors, and the expert reviewers in the field, so we could all work together to improve the manuscripts.’

After working as a scientific editor at Nature for 10 years, Lesley then started up a new side journal called Nature Communications. Unlike the main body of Nature manuscripts, Nature Communications published submissions from specialised research areas. The journal grew in a way that Lesley never imagined. Her original team of a mere 3 editors grew to 40 in just 6 years.

After leaving Nature Communications to start up her own independent business venture, Lesley would still definitely say that Nature Communications has been one of the highlights of her career. The journal received almost 1,900 submissions in her last month as Editor-in-Chief. Also, the journal achieved an impact factor of an impressive 12. Contextually, in the 2017 Journal Citation Report by Clarivate Analytics, which reviewed over 12,000 different journals’ impact factors, they found only the top 1.9 percent of journals received an impact factor of 10 or above.

Now, she manages her own freelance consulting company called Anson Scientific. While running her business she is able to rekindle with the primary scientific literature, as she is able to provide editing advice on scientific papers destined to be submitted for publication.

As the founder of Anson Scientific, Lesley has been able to edit publications from establishments such as Harvard Medical School and Imperial College London while also providing training for individual researchers and institutions in manuscript editing and writing.

Lesley’s career is an example of how opportunities arise and can steer you in a direction you never imagined. The important thing however, is that you keep in touch with your key interest, which for Lesley was the ever-evolving environment of science. The important thing however, is to remain in touch with your key interest, which for Lesley was the ever-evolving environment of science.

‘Do something you love, as you spend a lot of time doing it,’ Lesley concludes.

REFERENCES
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