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Article

Improving wound healing by breaking down biofilm formation and reducing nosocomial infections

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SUMMARY

A retrospective study that was published concluded that hospital admissions for adult patients in the United States suffering from skin infections is increasing by significant amounts. In a 10-year period in the early 2000's, infections due to *S. aureus* increased by 123%, and this number is increasing as time goes on [1]. The purpose of this experiment was to use hyaluronic acid, silver nanoparticles, and a bacteriophage cocktail to create a hydrogel. Unlike traditional hydrogels, which only aid in the protection and healing of wounds, this hydrogel promoted wound healing by increasing cell proliferation while simultaneously disrupting biofilm formation and breaking down *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are two strains of bacteria that attribute to nosocomial infections and are increasing in antibiotic resistance. The biofilm formation forms a barrier that gives the bacteria the power to resist antibiotics and give a pathway to create a source of systemic chronic infections [2]. If hyaluronic acid is used in conjunction with colloidal silver and species-specific bacteriophages, we predicted that cell proliferation in an in vitro wound healing model would increase. We tested our bacteriophage-containing hydrogel on human fibroblast cells and on *S. aureus* and P. aeruoginosa cultures to investigate its potential wound healing and antibacterial properties. In support of our hypothesis, we found that cell proliferation increased. Simultaneously, the bacteriophage cocktail and colloidal silver decreased biofilm growth in both *3* and *P. aeruginosa* while not affecting cell viability. This finding is important because it offers a cost-effective, non-invasive approach to improving wound healing without the use of antibiotics, which is important in a world where antibiotic resistance is becoming an increasingly prevalent problem.

INTRODUCTION

Pseudomonas aeruginosa is a gram-negative bacterium. Gram-negative bacteria are typically more resistant to antibiotics than gram-positive bacteria due to the fact that they have a unique outer membrane able to prevent antibiotics from penetrating the cells. Its natural habitat typically includes moist locations. *Pseudomonas aeruginosa* is considered an opportunistic human pathogen. Not only

can it cause both acute and chronic infections in patients that are immunocompromised or being treated for severe burns and injuries, but it is also both invasive and toxigenic [3]. These bacteria are able to survive in temperatures up to 41°C (105.8°F), can be transferred through environmental conditions, and frequently cause life-threatening infections in patients that have cystic fibrosis. [4].

However, drug-resistant gram-negative infections, caused by bacteria such as *Pseudomonas aeruginosa*, have emerged as major concerns in hospitals, nursing homes, and other healthcare settings. *P. aeruginosa* is a major cause of nosocomial infections which affect more than 2 million patients every year and account for around 90,000 deaths annually [5]. In some cases, the bacteria are able to enter the body through urinary and intravenous catheters, ventilators, or wounds and can lead to pneumonia and infections of the bloodstream, bones, joints, and urinary tract. These types of infections disproportionately affect the very ill and the elderly and are often difficult to treat [6].

Unlike *Pseudomonas aeruginosa*, *Staphylococcus aureus* is a gram-positive bacteria strain. Similar to *Pseudomonas aeruginosa*, it is a common cause of systemic infections, particularly if the cutaneous barrier is disrupted [7]. *Staphylococcus aureus* causes many types of infections, including superficial skin lesions, boils, styes, localized abscesses, and furunculosis. It is a dangerous infection due to three main virulence factors: surface proteins that promote colonization of the host, factors that inhibit phagocytosis, and the production of toxins that damage host tissues and cause disease symptoms [8].

Hydrogels are polymer networks composed of 90% water in a gel base. They create a moist healing environment that promotes granulation and epithelialization, which is the generation of new connective tissue and blood vessels that eventually leads to the closing of the wound [9]. Additionally, the hydrogel's high-water content cools the wound, producing pain relief that can last up to 6 hours [10]. Hydrogels also decrease patient discomfort when changing burn dressings because they do not adhere to the wound surface [11].

Bacteriophages are viruses that live within bacteria and self-replicate, eventually destroying the bacterial cell. They cannot reproduce alone, and they require the bacterial cell as a host to reproduce. The receptor structure on bacteriophages is so specific that a phage can only attack bacteria that has a cell surface that exactly "matches," therefore they are species-
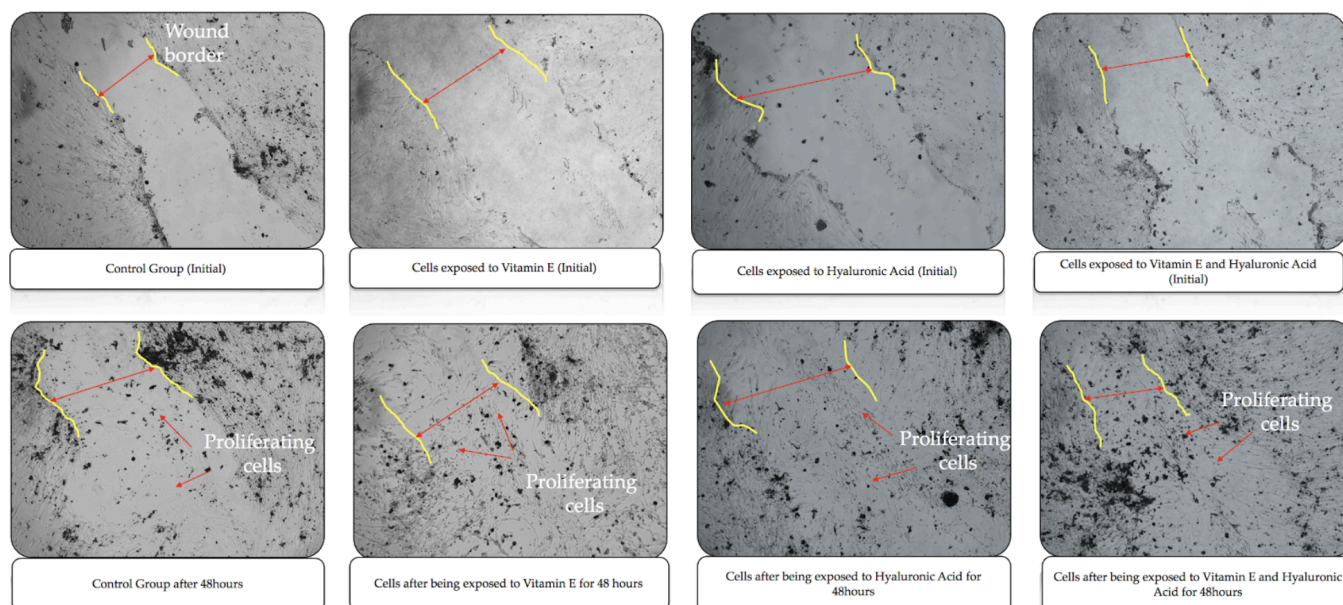
Figure 1: Hyaluronic acid and vitamin E enhance cell proliferation. The cell proliferation assay showed an increase in proliferation in the cells treated with hyaluronic acid, vitamin E, and both hyaluronic acid and vitamin E compared to untreated cells. Forty-eight hours post treatment, there is a significant difference in proliferation between the treatment groups and the control group. In the pictures of the control group, the borders of the wound can still be seen while in the pictures of the treatment group the borders of the wound have mostly given way to the proliferating cells.

specific. As bacteria evolves, bacteriophages also evolve so that they can increase in numbers and propagate over time. Due to their specificity, bacteriophages are an effective use for medicine because they can destroy antibiotic-resistant strains of bacteria. Literature has shown that treatments using bacteriophages began as early as 1921 [12].

As an element, silver is effective against more than 650 pathogens and has a broad spectrum of activity [13]. As such, it has a high potential for solving the problem of multidrug resistance, which is observed in several bacterial strains. When used as a nanoparticle, silver's antimicrobial properties are enhanced; this is because silver particles have a larger surface area allowing for the bacteria to be exposed to more silver, though the antibiotic mechanism is currently not understood [14]. This allows silver to be used in a wider range of applications, including wound dressings and surgical devices [15]. It has the ability to inhibit both enzymatic systems in the bacteria and alter the synthesis of DNA. Multiple studies have demonstrated silver nanoparticles' effectiveness at preventing bacterial growth and development [16].

Hyaluronic acid is the main component of the extracellular matrix and is considered one of the key factors in the tissue regeneration process. It is present through all steps of the wound healing process as a factor that actively modulates tissue regeneration [17]. Many studies investigating hyaluronic acid have demonstrated its ability to modulate inflammation, cellular migration and angiogenesis [17]. Adding excess hyaluronic acid to a wound speeds up the recovery process of the wound and is considered a safe and effective approach to treat wounds [18].

Vitamin E has been shown to regulate cellular signaling and gene expression and influence wound healing [19]. It defends cellular membranes from reactive oxygen species by activating various signal transduction pathways and can be classified as an antioxidant. It also modulates the expression of connective tissue growth factor, thereby protecting wounds from infections such as *Staphylococcus aureus* infections and *Pseudomonas aeruginosa* infections [20]. Thus, we predicted that using hyaluronic acid and Vitamin E in conjunction with colloidal silver and species-specific bacteriophages would increase cell proliferation in an in vitro wound healing model.

The purpose of this experiment is not only to use hyaluronic acid, Vitamin E, silver nanoparticles, and a species-specific bacteriophage cocktail to create a hydrogel, but to test its beneficial wound-healing capabilities and antibiotic properties. Unlike traditional hydrogels, which only aid in the protection and healing of wounds, this hydrogel is not only able to protect and heal wounds, but it would be able to heal wounds and burns by increasing cell proliferation while simultaneously disrupting biofilm formation and breaking down harmful bacteria strains that cause systemic nosocomial infections, therefore providing a more efficient wound-healing mechanism.

RESULTS

In the cell proliferation assay, the cells exposed to Vitamin E, hyaluronic acid, and the combination of Vitamin E and hyaluronic acid all showed an increase in proliferation of the human dermal fibroblasts over the control group, almost reaching confluency in a two-day period. Simultaneously, the assay run to test for a decrease in bacterial growth and cell viability yielded results that showed silver nanoparticles are
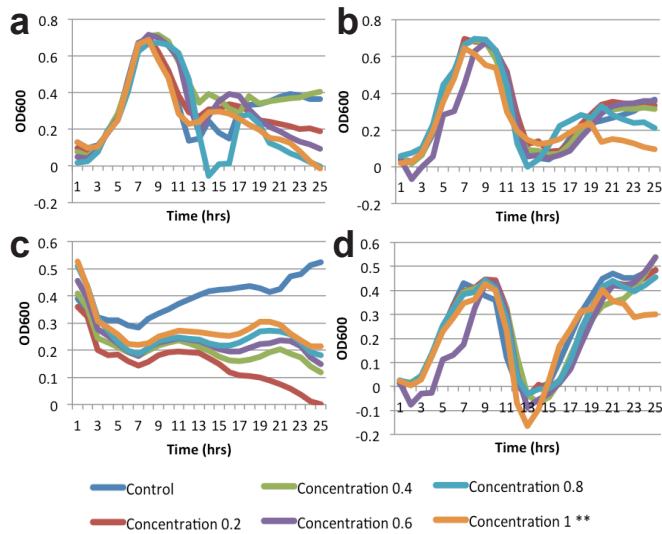
Figure 2: The effects of silver nanoparticles on bacteria growth. (A) There was a significant decrease in growth of *Staphylococcus aureus* when exposed to an 80% concentration of a 0.02 mg/mL stock solution of 40 nm silver nanoparticles (p < 0.05 and an 0.02 mg/mL stock solution of silver nanoparticles (p < 0.05). (B) There was a significant decrease in growth of *Staphylococcus aureus* when exposed to 0.02 mg/mL stock solution of 40nm silver nanoparticles. A one-tailed T-Test was performed to test for statistical significance and the only p-values less than 0.05 was the p-value for the 100% concentration. (C) There was a significant decrease in growth of *Pseudomonas aeruginosa* when exposed to all concentrations of 20nm silver nanoparticles (p < 0.05). (D) There was a significant decrease in growth of *Pseudomonas aeruginosa* when exposed to 40nm silver nanoparticles. (p < 0.05 for 100% concentration, p > 0.05 for all others).

able to decrease the growth of both *P. aeruginosa* and S. aureus by significant amounts without a significant effect on the viability of the human dermal fibroblasts.

In the cell proliferation assay (**Figure 1**), a clear difference could be seen between cell proliferation into the wound of the control group and cell proliferation into the wound of experimental groups, even within 24 hours of treatment. By 48 hours of treatment, the three experimental groups had almost reached confluency while the control group still showed the outline of the scratch. Not only this, but the control group still had many clear spaces and showed minimal cell movement. The cells exposed to both hyaluronic acid and Vitamin E had the most proliferation, but individually the cells exposed to vitamin E exhibited more proliferation than the cells exposed to hyaluronic acid. There were three trials per experimental group and each group showed similar results.

Due to some literature suggesting that 20nm silver nanoparticles were small enough to enter human cells and cause harm [21], we used both 20nm and 40nm silver nanoparticles. When we ran an OD600 assay using the silver nanoparticles, the results showed that there were concentrations in each experimental group that were statistically significant in reducing the amount of bacteria present (**Figure 2**). We performed a one tailed T-Test to test for statistical significance and there was a comparison between

a normalized initial concentration and then the corrected final concentration of bacteria present to determine which group was statistically significant in reducing the amount of bacterial growth.

The cell viability assay showed that both the 20nm and 40nm silver nanoparticles did not affect cell viability by a statistically significant amount in any of the groups (**Figure 3**; p = 0.0622 for the lowest percent viability, all else were larger, one-tailed T-test). The lowest percent viability was the treatment with undiluted 0.02 mg/mL silver nanoparticles of both 20nm and 40nm silver nanoparticles together, which still had a viability above 80%. This is important because it suggests that while the silver nanoparticles are able to break down bacteria, they do not display short term negative effects on the body. In the parts of the experiment where silver nanoparticles are tested solely on bacteria, each trial had one or more concentrations that reduced the bacterial population by a statistically significant amount (**Figure 2**). In the cell viability assay there were three trials per experimental group and each group showed similar results. A one tailed T-Test was performed to compare the differences in the viability of the experimental groups to the control groups.

After isolation, purification, and propagation of the bacteriophages were complete, 7 bacteriophages for *P. aeruginosa* and 5 bacteriophages for S. aureus were isolated from the samples collected from the Broward Sewer Treatment Plant. This constituted a bacteriophage cocktail of 12 individual bacteriophages that was specific to S. aureus and *P. aeruginosa*. When an OD600 assay was run using the bacteriophage cocktail, all concentrations significantly reduced the number of bacteria present in the solution (p = 0.0191, one-tailed T-test) and did not have an effect on the cells (p = 0.117; **Figure 3**). Ultimately, the results of these experiments support our hypothesis that the use of hyaluronic acid and vitamin E in conjunction with colloidal silver and species-specific bacteriophages increases cell proliferation in an in vitro wound healing model while decreasing bacterial growth.
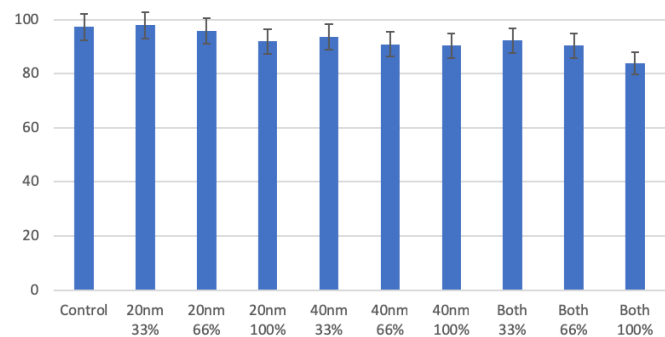

Figure 3: Silver nanoparticles do not affect HDF viability. The results of the cell viability assay show that both the 20nm and 40nm silver nanoparticles do not have a statistically significant effect on the viability of the human dermal fibroblasts (p > 0.05).

DISCUSSION
Application

Each year in the United States, over 2 million people develop an antibiotic resistant infection and roughly 23,000 people die due to infections from antibiotic resistant strains of varying types of bacteria [22]; the findings of this project have implications in ultimately reducing that number. The results offer a cost-effective, non-invasive approach to improving wound healing without the use of antibiotics, therefore not contributing to antibiotic resistance.

By using silver nanoparticles and species-specific bacteriophages (**Figure 2A, 2B, 2C, 2D**), the hydrogel created in this project targets strains of bacteria that have the potential to adapt to antibiotics without affecting the viability of the cells, increasing wound-healing efficiency. Moreover, the use of bacteriophages ensures that the hydrogel does not exacerbate antibiotic resistance, providing solvency to a problem that has increasingly plagued the medical community. In essence, our experiment demonstrated that both the silver nanoparticles and the species-specific bacteriophage cocktail are able to significantly reduce the number of bacteria present, thereby having implications in decreasing hospitalized patients' risk of infection while simultaneously circumventing the issue of antibiotic resistance.

The scratch assay (**Figure 1**), which used both hyaluronic acid and vitamin E, showed that both substances were able to decrease the time for new cells to proliferate into a wound after a confluent layer of cells has been disrupted. Therefore, hyaluronic acid and vitamin E reduce the healing time and could potentially decrease the chances of infection in hospitalized patients., thereby enhancing hospital efficiency. In addition, by simply using a hydrogel, the discomfort of the patient can be decreased for hours at a time.

Another benefit of our approach is that the hydrogel can be customized to benefit the patient or the hospital. If there is a certain antibiotic resistant strain of bacteria present in the hospital, bacteriophages for that specific strain can be isolated from local sewer treatment plants or even the patients and added to hydrogel for a more targeted and specific treatment.

In summary, the results of this experiment provide conclusive support of a more efficient mechanism for treating patients by decreasing the time of hospital stay, undermining antibiotic resistance, and diminishing the presence of nosocomial infections. This multifaceted approach targets multiple problems that the medical community faces and makes for a more successful healthcare system.

Limitations

Originally, we planned on gene-sequencing the bacteriophages to show genotypic variation between bacteriophages. Due to the lack of availability of primers to sequence the bacteriophages collected in the experiment, we were not able to gene-sequence the phages.

Another limitation is that the experiment was performed in a 12-well plate using a single cell type as a model for humans, a much more complex recipient. Due to this, the effects of the project were limited and cannot predict how the hydrogel would react when exposed to multiple layers of skin as well as blood and inflammation. While prior research and other experiments have suggested that such limitations would not negatively impact the implications of this experiment [citations needed for this prior research], the lack of human trials leaves room for uncertainty. Small molecular differences may seem minor, but these minor molecular differences could cause a cascade effect in an in vivo system.

Due to the absence of biokinetics, it can only be hypothesized that the hydrogels' success in an in vitro model will translate to success in an in vivo system. Therefore, this in vitro wound healing model can simply lay the groundwork for further research where it will eventually be tested in an in vivo system.

Future Research

In the future, we would like to test the hydrogel on a 3D burn model created using collagen, fibrin, human foreskin fibroblasts, and human epithelial keratinocytes due to its success in increasing proliferation on human dermal fibroblasts. Such an experiment would provide insight into how hyaluronic acid and vitamin E would affect proliferation in multiple cell types.

Also, we would like to test the hydrogel on tissue samples from various organisms. This will allow us to test the hydrogel on models most similar to the human skin. In addition, we would like to experiment with multiple injury models to determine how effective the hydrogel is at increasing the proliferation of multiple cell types in a wound affecting multiple layers of the skin.

We would also like to expand the experiment beyond the scope of the two bacterial strains used here and create a more all-encompassing bacteriophage cocktail. Such a treatment would allow for a more systemic, multi-faceted approach to wound care by addressing a widespread variety of bacterial infections that plague hospitals on a global scale.

MATERIALS & METHODS
Plating Cell Culture

The external portion of a vial of Human Dermal Fibroblasts: HDF, adult (Sigma-Aldrich 106-05A) was decontaminated using 70% alcohol in a sterile biological safety cabinet and the cells were resuspended in the vial by gently pipetting the cells 5 times with a 2 mL pipette. The cell suspension was pipetted from the vial into a T-75 flask containing 15 mL of fibroblast growth medium (Sigma-Aldrich 116-500) and placed in a 37oC, 5% CO2 humidified incubator. The fibroblast growth medium was changed every other day until the cells covered roughly 60% of the plate and were split onto new plates when the cells reached 80% confluency. [23]

Isolation of Phages from Environmental Samples

10 mL of nutrient broth (20 g nutrient broth

(ThermoScientific)) was inoculated with a single colony of *Pseudomonas aeruginosa* and incubated overnight at 37°C. The next morning, 0.5 mL of 10x nutrient broth (20 g nutrient broth (ThermoScientific), 50 mM CaCl2, 50 mM MgCl2, 100 mL dH20) was added aseptically to a sterile glass bottle along with 4.5 mL of 0.2micron filter-sterilized sewage samples, obtained and given to us by the North Regional Water and Wastewater Treatment Plant, and mixed well. The broth was inoculated with 1 mL of the overnight culture and incubated overnight at 37°C. The next day, nutrient agar plates were labeled to distinguish between the control. *P. aeruginosa*, and S. aureus. The required number of agar overlays (2 g nutrient broth (ThermoScientific), 10 mL 50 nM CaCl2, 10 mL 50 nM MgCl2, 80 mL H20, 0.2 g molecular-grade agarose) were prepared and cooled to 45°C. Once cooled, 200 µL of overnight culture was added to an overlay. It was swirled gently and poured over the surface of the appropriately labelled nutrient agar plate. The plates were then incubated at 37°C for 6h. For experimental samples, 1 mL of the overnight sewage–bacteria culture was centrifuged at 13,000 rpm for 5 min. 100 µL was spotted onto the center of a set overlay and the remaining supernatant was stored at 4°C in a sterile Eppendorf. If phages are present in the sample, a zone of clearing or individual plaques will appear in the center of the plate (**Figures 4 and 5**). [24]

Propagation of Phages from Environmental Samples

10 mL of nutrient broth were inoculated with a single colony of the host strain and incubated overnight at 37°C. Using the supernatant from the "Isolation of Phages from Environmental Samples" protocol, a dilution series of the sample in sterile nutrient broth was prepared from 1:10 to 1:107. The required number of overlays were prepared and cooled to 45°C. Once cooled, 200 µL of overnight culture was aseptically added to an overlay along with 20 µL of the diluted phage sample. The overlay was gently swirled and poured over the surface of the appropriately labelled nutrient agar plate. The plate was gently pushed back and forth so that the overlay completely covered the surface of the agar plate. The plate was transferred to the 37°C incubator and incubated for 6h with the plate lid facing upward. If phages are present in the sample, individual plaques will be seen in the lower dilutions (**Figures 4 and 5**). The higher dilutions will likely be completely lysed. [24]

Purification of Phages from Environmental Samples

Plates from the "Propagation of Phages from Environmental Samples" protocol were inspected to asses plaque distribution. From the plates with well-separated individual plaques, a sterile inoculation loop was used to carefully scoop off a single plaque into 1 mL sterile nutrient broth in a 1.5 mL Eppendorf tube. The sample was vortexed and stored at 4°C for at least 2h. From the previously prepared plaque suspension, a dilution series was created where in tube 1, 20 microliters of supernatant were added to 180

Figure 4. Photographs of *P. aeruginosa* Bacteriophages. Photos of **(A)** 10^{-1}, **(B)** 10^{-2}, **(C)** 10^{-3}, and **(D)** 10^{-4} dilutions of the bacteriophage cocktail used to treat *P. aeruginosa*. Unlike the dilutions performed in the isolation, propagation, and purification stage in the procedures, these images show serial dilutions of the bacteriophage cocktail that were tested in the OD600 assay. Note that the 10^{-1} dilution has no bacteria, but as the dilutions progress, more bacteria can be seen (the bacteria appears as cloudy while the clearings are the areas phages destroyed the bacteria.)

Figure 5. Photographs of *S. aureus* Bacteriophages. Photos of **(A)** 10^{-1}, **(B)** 10^{-2}, **(C)** 10^{-3}, and **(D)** 10^{-4} dilutions of the bacteriophage cocktail used to treat *P. aeruginosa*. These images show serial dilutions of the bacteriophage cocktail that were tested in the OD600 assay. Note that the 10^{-1} dilution has no bacteria, but as the dilutions progress, more bacteria can be seen (the bacteria appears as cloudy while the clearings are the areas phages destroyed the bacteria.)

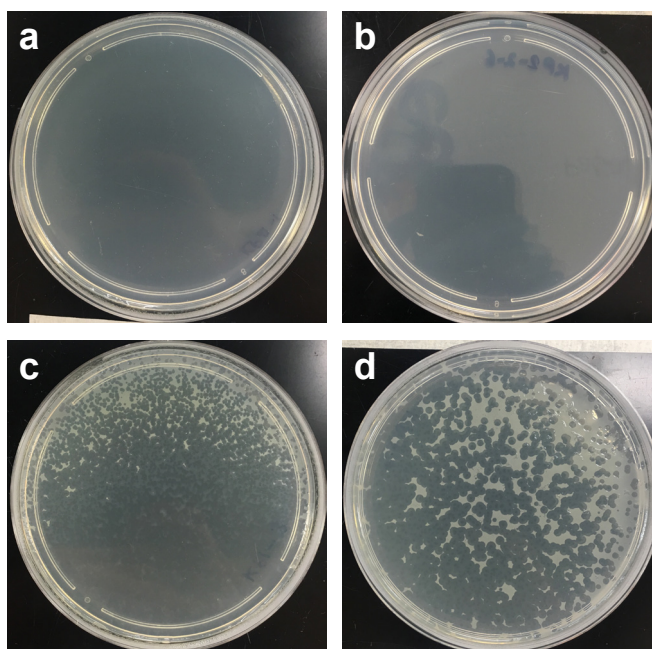microliters of nutrient broth and then in tube 2, 20 microliters of tube 1 were added to 180 microliters of nutrient broth, until you got to tube 10.. For each plaque type, the plaque picking process was completed three times until a pure phage sample was created, this is when all phages on the plate are the same size/shape and visually display the same structure. [24]

Cell Viability

Human dermal fibroblasts were taken out of T-75 flasks, and equal amounts of cell suspension were then plated in a 24 well plate. After letting the cells reach confluency, human dermal gibroblast growth medium was supplemented with varying amounts of 20nm, 40nm, and a combination of both 20 and 40nm silver nanoparticles made with the fibroblast growth medium [25]. After a 24 hour period of incubation with the silver nanoparticles, the cells were counted.

Cell Counting

The T-75 flasks from the "Cell Viability" protocol were gently swirled to ensure that the cells were evenly distributed. Before the cells had a chance to settle, 0.5 mL of cell suspension were removed using a 5 mL sterile pipette and placed in an Eppendorf tube. 100 μL of cells were pipetted into a new Eppendorf tube and 400 μL 0.4% Trypan Blue (final concentration 0.32%) was added to the solution. Using a pipette, 100 μL of Trypan Blue-treated cell suspension were removed from the Eppendorf tube and placed in the hemocytometer. A microscope with a 10X objective was used to focus on the gridlines of the hemocytometer. Using a counter, the live, unstained cells were counted in one set of 16 squares. The hemocytometer was then moved to the next set of 16 corner squares and this process was repeated until all 4 sets of 16 corners were counted. The average cell count from each of the sets of 16 corner squares were multiplied by 10,000, and then multiplied by 5 to correct for the 1:5 dilution from the Trypan Blue addition. The final calculation was the number of viable cells/mL in the original cell suspension. To calculate viability, the live and dead cell counts were added to obtain a total cell count. Then, the live cell count was divided by the total cell count to calculate the percentage of viable cells [26].

Cell Proliferation Assay

Equal amounts of cells were transferred from T-75 flasks to each of the wells in a 24-well plate. After letting the cells reach confluency, a scratch assay was performed by taking a sterile pipette tip and dragging it across the center of the well, stimulating a wound [27]. Varying concentrations of a 1% stock solution of hyaluronic acid, varying concentrations of a 36mg/ml stock solution of vitamin E, and a combination of the two were added to the wells. Over a two-day period, proliferation into the wound was recorded and images were taken.

OD600 Assay

The OD600 method was performed automatically in a high throughput manner using a microtiter plate reader over a 24-hour period. 1/10 dilutions of both *P. aeruginosa* and S. aureus were taken from active cultures that were stored at 4˚C and a 12-well multichannel pipette was used to pipette equal amounts of bacteria into the wells of a 96-well plate. The control was set aside as row A in the 96-well plate, and various concentrations of a stock solution of 0.02 mg/mL silver nanoparticles, of both 20nm and 40nm solutions, were made. These solutions were then pipetted into the rows and placed into the spectrophotometer that was set to 37°C. The program was set to run for 24 hours and take readings every hour [28].

Statistics

To test for significance in decrease in the experimental groups compared to the control group for the OD600 assay, the Cell Viability Assay, and the Cell Counting Assay, a 1 Tailed T-Test was performed comparing each experimental group to the control group.

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Misconceptions Regarding Blindness are Prevalent: Possible Implications for Best-practices and Policy Making

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ABSTRACT

The intention of this study was to investigate the perceptions that sighted people have about blindness and what the visually impaired perceive as the most challenging aspects of being blind. Sighted subjects were given surveys regarding general facts about blindness as well as questions about what they consider to be the most pressing difficulties faced by the blind. Blind subjects were also given surveys that asked them about what they perceive to be the greatest difficulties that they face, conditions that have improved for them, and what changes should be made to improve their lives. We hypothesized that sighted participants would be at least 50% accurate on routine questions about blindness, that blind participants would more often report difficulties in accomplishing daily tasks such as driving and reading as their greatest challenges, and that adults would be more knowledgeable about blindness than minors because of their increased level of education and age. The results from this study suggest that the sighted have significant factual misunderstandings about blindness as well as what should be done to help the blind. The results also demonstrate that blind subjects surveyed for this study find the most challenging adversity to be the lack of awareness and understanding about their circumstances. Additionally, sighted minors and sighted adults did not have a significant difference in their general knowledge about blindness.

INTRODUCTION

According to the International Classification of Diseases - 10 (Update and Revision 2016), vision function is classified into 4 broad categories: normal vision, moderate vision impairment, severe vision impairment, and blindness (1). Moderate and severe vision impairment are grouped under the term low vision, while blindness can be further divided into legal blindness and total blindness. Legal blindness is a level of vision loss that has been legally defined to determine eligibility for benefits. In the United States, this refers to a medically diagnosed central visual acuity of 20/200 or less in the better eye with the best possible correction, and/or a visual field of 20 degrees or less (6). Total blindness refers to an inability to see anything with either eye (6). Together, low vision and blindness groups represent all vision impairment (5).

According to the World Health Organization, an estimated 253 million people around the world live with visual impairment, and about 36 million of these people are legally blind (2). 10-15% of these legally blind people are totally blind (7). It is estimated that the number of people with vision impairment could triple due to population growth and aging (2). The global leading cause of blindness is cataracts (2). Other major causes of visual impairment are glaucoma, age-related macular degeneration, corneal opacities, diabetic retinopathy, childhood blindness, trachoma, and onchocerciasis (3). Excluding age-related macular degeneration, it is estimated that about 80% of all visual impairment is avoidable (4).

While medical and social care programs have been available to assist the blind, public policy relating to blindness has lagged behind. Studies have shown consistently high unemployment rate (9), depression rate (12), rates of bullying and other harassment (10) experienced by the blind.

This study was designed to assess perceptions about blindness by subjects with vision and blindness. A survey was given to subjects with vision to assess their general understanding of blindness. A second survey was given to blind subjects inquiring about their causes of blindness, what they find most challenging, and what they consider to be the most meaningful changes that would improve their lives.

Before conducting the surveys, we hypothesized that sighted participants would be at least 50% accurate on routine questions about blindness because previous studies have shown the accuracy of public perceptions about the understanding of blindness to range from 50-75% (8). We also hypothesized that blind participants would more often report difficulties in accomplishing daily tasks, including driving and reading, as their greatest challenges. Our final hypothesis was that adults, because of their increased level of education and age, would be more accurate than minors in their understanding about blindness.

RESULTS

The authors wanted to assess (1) perceptions about blindness by subjects with vision and blindness, (2) determine what blind participants consider most challenging tasks, and (3) if a higher level of education and age translates to a more accurate understanding about blindness. To assess this we designed a survey for subjects with vision to assess their general understanding of blindness. A second survey was also given to blind subjects inquiring about their causes of

blindness, what they find most challenging, and what they consider to be the most meaningful changes that would improve their lives. The data was then analyzed to determine if a statistically significant difference in an understanding about blindness between adults and minors could be demonstrated.

Blind subjects consider poor acceptance or lack of understanding as greater challenges than an inability to perform any individual task **(Table 1)**.

What is the most difficult part of being blind?	Percentage of blind respondents answering
Unfair Treatment	66%
Not being able to read	22%
Not being able to drive	18%
Lack of independence	14%
Not knowing where things are	10%
Not being able to recognize people	8%
Other	8%

Table 1. Blind individuals' perceptions about their greatest struggles.

Sighted individuals have very little knowledge about blindness and the difficulties that the blind face. 66% of blind individuals felt that unfair treatment was the most difficult part of being blind. Inability to do specific tasks such as reading (22%), driving (18%), being independent (14%), locating things (10%), recognizing people (8%), not being able to go shopping independently, color-coordinate their outfits, or enjoy artwork (8%) were not reported as major difficulties. Most blind respondents reported that technology (28%) and transportation (16%) have become much more accessible to the blind, but that increased awareness about blindness (8%), employment (4%), finding friends (4%), and having other resources (4%) have remained difficult issues **(Table 2)**.

What has become easier for blind people?	Percentage of blind respondents answering
Technology	28%
Transportation	16%
More education about blindness	8%
More braille	4%
Employment	4%
Finding friends	4%
Other	4%

Table 2. Blind individuals' perceptions about what has become easier for them.

Most of the blind individuals (24%) surveyed in this study reported that an increase in awareness and education about blindness would most greatly benefit them. Others reported that better transportation (12%), medical advancements (8%), increased accessibility (4%), more money for research on blindness (4%), blind people's awareness of the resources available to them (4%), or other improvements (8%) would most greatly improve their lives **(Table 3)**.

What change should be made to improve the lives of the blind?	Percentage of blind respondents answering
More education about blindness	24%
Better transportation	12%
Medical advancements	8%
Other	8%
Increased accessibility	4%
More money for research on blindness	4%
The blind should be more aware of the resources available to them	4%

Table 3. Blind individuals' perceptions on what can be done to improve their lives.

Minors had the most success answering "What is the most difficult part of being blind?" (58% correct) and had the most difficulties answering "How many people around the world suffer from blindness?" (21% correct) **(Table 4)**. Adults had the most success answering "What is the global leading cause of blindness?" (53% correct) and had equal difficulties with "How many people around the world suffer from blindness?" and "What percentage of global visual impairment is avoidable?" (26% correct) **(Table 4)**.

Question	Answer	% of correct answers overall by sighted participants	% of sighted minors who answered correctly	% of sighted adults who answered correctly
How many people around the world suffer from blindness?	30-40 million	23.3%	20.8%	26.3%
What is the global leading cause of blindness?	Cataracts	44.2%	37.5%	52.6%
What percentage of global visual impairment is avoidable?	80%	30.2%	33.3%	26.3%
What percentage of legally blind people can not see anything?	10%-20%	41.9%	41.7%	42.1%
According to the U.S. Equal Employment Opportunity Commission, what percentage of blind people are unemployed?	63.2%	39.5%	41.7%	36.8%
What is the most difficult part of being blind?	Not being accepted/ understood	48.8%	58.3%	36.8%
Which of the following positive changes has had the greatest impact on the blind?	Better transportation and technology options	32.6%	25%	42.1%

Table 4. Sighted individuals' responses to the survey questions.

Overall, minors answered an average of 36.3% of the questions correctly while adults answered an average of 39.1% of the questions correctly **(Table 5)**. The median correct score was 42.9% for the minors and adults **(Table 5)**. There was no significant difference between the performance of sighted minors and sighted adults on this survey (p-value 0.94) **(Figure 1)**.

	Minors	Adults	Total
Sighted participants in study	24	19	43
Mean score	36.3%	39.1%	36.9%
Range of scores	6	5	6
Median score	42.9%	42.9%	42.9%
Sample standard deviation	1.59	1.57	1.56
Sample Variance	2.52	2.47	2.44
Standard Error	0.324	0.360	0.238

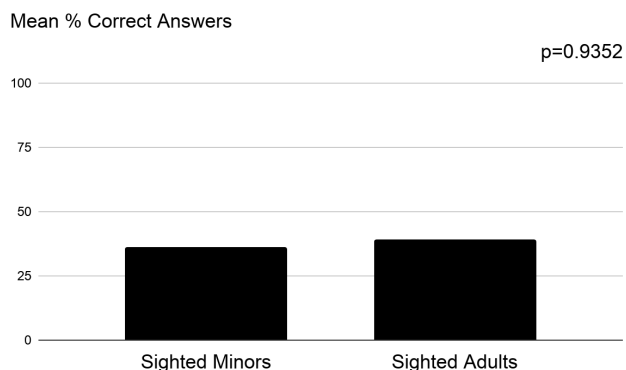**Table 5.** Comparison of sighted minors and adults' performance on the survey.

Figure 1. Mean scores for sighted adults and minors.

DISCUSSION

This study demonstrates that sighted participants do not have a reasonably accurate understanding about blindness. Evidence was not found to support the 3 hypotheses of this study – that sighted participants would have a reasonably accurate (50%) understanding about blindness, that blind subjects would consider difficulties in accomplishing daily tasks as their greatest challenges, and that sighted adults would score higher on the survey than sighted minors. Evidence from our study shows that sighted individuals have limited knowledge about blindness (about 35% - 40% accuracy). Additionally, we found that social circumstances such as poor acceptance and lack of understanding have greater negative effects on the blind than the inability to do daily tasks that require vision. Lastly, there was no statistically significant difference in the performance accuracy between sighted minors and sighted adults on a questionnaire about blindness (p-value 0.94).

These results have implications for best-practices and public policy making decisions. Public policy relating to blindness remains inadequate as demonstrated by the high unemployment rate (9), depression rate (12), and rates of bullying and other harassment experienced by the blind (10). The causes are likely multifactorial, but could reflect the fact that very few blind people are involved in policy making and significant misperceptions about blindness are prevalent among the sighted who make these policies.

The unemployment rate for the blind and visually impaired is 63% and remains the highest among any subgroup of population, even among those with disabilities (9). This is not because of a lack of skill or other inferiority when compared to other candidates. Multiple reasons have been cited, including lack of employer awareness of state and federal tax incentives, vocational rehabilitation services, and aides available to blind employees (9). Additionally, employers may falsely assume that accommodations would be too costly, that liability would increase, or that the blind are not dependable workers (9).

Despite laws to prevent employers from discriminating against the blind, many employers allow their misperceptions about the capabilities of the blind to override these laws. As a result of these concerns, unemployment rates for the blind and visually impaired have remained at least in the 60 - 65% range (9). Not only are many qualified blind people left unemployed, but they also often feel incapable or inferior, feelings which are linked to mental health disorders such as depression (10). According to the Anxiety and Depression Association of America, approximately 6.7% of American adults suffer from depression (11). By comparison, recent studies suggest that approximately one-third of visually impaired older adults experience mild but clinically significant symptoms of depression and anxiety (12). It can be reasonably assumed that poor employment and financial well-being, discriminatory attitudes towards the blind, and the physical limitations of blindness contribute significantly to the large number of people with blindness who suffer from depression.

Our study relied on surveys to collect data and the findings may be limited by sample size, self-selection of subjects, and missing data from some participants. Thus, external validity, or the extent to which the research findings can be generalized to other similar populations, may be a concern with this study. Therefore, a future study could use a larger sample size and analyze how factors such as geographical region, gender, socioeconomic status, or level of education affect knowledge and awareness about the blind.

This study highlights the lack of awareness about blindness. The findings also demonstrate that blind subjects perceive the lack of acceptance and understanding as their biggest challenge rather than difficulties in performing routine daily tasks. Previous studies have shown that after appropriate training sessions, sighted students' perceptions of blind students were more correct than those found in a pretest, suggesting that stereotypes and attitudes toward blindness can be changed with effective educational interventions (13). Misconceptions and poor understanding of blindness could be reduced with educational campaigns directed towards school-age children. Addressing these concerns and more research studies to determine the causes of these disparities are necessary.

METHODS

Permissions and Participants

Before this study was conducted, an SRC/IRB approval form was completed. Blind participants for this study were members from the Florida Outreach Center for the Blind in West Palm Beach, Florida. All blind participants gave verbal consent and were made aware of the intention and purpose of the survey. The 43 sighted participants were comprised of 24 minors and 19 adults. In this study, the mean age of the minors was 16 and adults was 54. The sample of sighted minors included high school students and other local community members. The sample of sighted adults included family members and friends. All sighted individuals that participated gave verbal consent and expressed their understanding of the intention and purpose of the study.

Survey Design and Testing

The blind participants were taken individually to a private room where they were verbally asked the questions in **Figure 2** and their responses were recorded verbatim.

Shivani Kumar 2018 Blindness Survey

Name:
Age:
Gender:
Race/Ethnicity:
Cause of Blindness:
How long have you been blind?

What do you feel are the most difficult parts of being blind?

What are some things that you feel have become easier for blind people?

What change(s) do you feel should be made to improve the lives of the blind?

How do you define blindness?

Figure 2. Survey given to blind individuals

The sighted students were given the survey in **Figure 3** on paper during class and were asked to write their responses. The students were asked not to reveal or discuss their answers with other students in the class until the entire survey process was over. All students from the school who participated in the study began the survey at the same time to reduce conversations about the survey before everyone was finished. Other minors in this study were surveyed over the phone and their responses were recorded verbatim. The sighted adults in this study were either given a hard copy of the survey to fill out or were surveyed over the phone to have their responses recorded verbatim. All blind participants in the study were residents of the state of Florida in the United States. Most of the sighted participants in the study claimed to be residents of the state of Florida in the United States, but some were residents of the states of Pennsylvania or New York. The data was analyzed with using an independent samples t-test.

Shivani Kumar 2018 Blindness Survey

Name:
Age:
Gender:
Race/Ethnicity:
Level of education:

How many people around the world suffer from blindness?
  A. 10-20 million
  B. 20-30 million
  C. 30-40 million
  D. More than 40 million

What is the global leading cause of blindness?
  A. Trachoma
  B. Retinitis Pigmentosa
  C. Cataracts
  D. Glaucoma

What percentage of global visual impairment is avoidable?
  A. 15%
  B. 35%
  C. 60%
  D. 80%

What percentage of legally blind people can not see anything?
  A. 1%-10%
  B. 10%-20%
  C. 40%-50%
  D. More than 50%

According to the U.S. Equal Employment Opportunity Commission, what percentage of blind people are unemployed?
  A. 21.4%
  B. 39.7%
  C. 63.2%
  D. 86.3%

What is the most difficult part of being blind?
  A. Not being able to read
  B. Not being accepted/understood
  C. Not being able to drive
  D. Not being able to see the people you love

Which of the following positive changes has had the greatest impact on the blind?
  A. Greater awareness and acceptance
  B. Better transportation and technology options
  C. Medical advancements that reduce the chance of blindness
  D. Laws that protect blind employment

How do you define blindness?

Figure 3. Survey given to sighted individuals.

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The role of recipients' race, gender, and species as incentives for charitable giving

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SUMMARY

While most not-for-profit organizations (NFPOs) find it challenging to raise funds and generate public interest in their causes, NFPOs focused on the welfare of animals seem to face even less support from society. This idea motivated us to investigate what factors drive individuals to help someone or something. Specifically, we tested the hypothesis that individuals support causes that are similar to themselves in terms of race, gender, and species. If this hypothesis were true, it could explain why animal-focused organizations have a harder time fundraising than organizations focused on helping humans. It would also have implications on how NFPOs should define target sponsor segments and efficiently use their resources in fundraising. Overall, we found that our participants favored causes that were closely associated with their race, gender, and species profile. We were able to statistically support the assertion that white Spanish subjects from Madrid overwhelmingly prefer charities aimed at white Spanish children instead of black African children. With respect to gender, our data overwhelmingly support the hypothesis that women prefer charities aimed at supporting a women's health issue versus a men's health issue. Our results with respect to men favoring men's health issues versus women's health issues were inconclusive. Finally, our data shows that overall, preferences for animal charities are lower than for charities supporting humans.

INTRODUCTION

In 2017, a total of $410 billion was donated to charitable causes in the United States, an increase of 3% inflation-adjusted dollars from 2016 (1). Outside of the United States the charity market is also changing.

According to DAFNE (Donors and Foundations Network in Europe), there are over 129,000 not-for-profit foundations (NFPOs) in Europe, most of which are relatively young (2). For example, more than 70% of Germany's foundations were established after 1990 and in Spain, 69% of foundations are under 18 years old (2). The global south has also experienced growth in the non-profit sector, although more inconsistently (2). The recent growth in the total amount of money donated to charities is due to several factors, including an increase in individual wealth and smaller family sizes, which lead to individuals who can afford to donate large sums (2).

In the United States in 2017, education was the most popular charitable cause (14% of donations) followed by human services charities (12%) (1). Even though donations to animal and environmental charities increased, they only made up 3% of the total 2017 United States donations (1). Internationally, education was the most popular cause, followed by health and social services.

Why is this the current situation? Why are certain causes more popular than others? It is clear that people's preferences are developed in very complicated ways. Context seems to play a big role. While religious charities are the most popular for baby boomers in the United States, millennials prefer to support children- and youth-related causes (3). Age likely plays a role in determining people's preferences. One's nationality also exerts an influence. A 2017 study found that for North Americans, the most popular charities were religious-based, contrary to Asia where children- and youth-related charities were most popular (3).

Other studies show that individuals want to fit into the norms of their social groups when deciding on donating to charitable causes. The UK Behavioural Insights Team demonstrated that communicating charity norms of a subject's profile (such as race and gender) increased average contributions (4). That is, when it comes to making charitable donations, individuals desire to follow the norm of their profile group (4). Another study by Croson and Shang demonstrated that revealing similarities between a prospective donor and the profile of current donors increased the probability and overall amount of donation by the prospective donor (5). In summary, individuals are more likely to make higher donations to causes that are supported by other individuals with the same profile characteristics (race, gender, etc.).

There is a wide body of research aimed at understanding individual motivation driving charity. In the work, "Behavior and Charitable Giving," the authors outline much of the research performed to understand individual behavior and therefore identify the strategies that can improve charity fundraising (6). We learn that individuals are influenced by the charitable actions of their social peers when making their own donation decisions, so a charity can boost fundraising by stating that others of similar race, income, social status, and gender support it. We also learn that providing specific examples of the benefits of a charity group also increases the possibility of raising funds (6). To add to this existing body of knowledge, it is important to understand whether demographic similarities between donors and recipients also motivates charitable giving.

Without contradicting previous studies on the behavior and motivation of charitable giving, we proposed that the
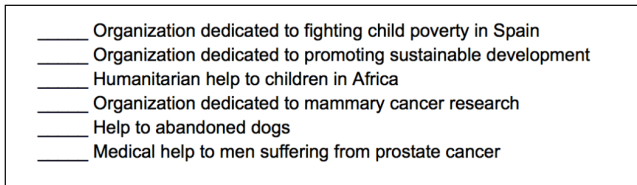
_____ Organization dedicated to fighting child poverty in Spain
_____ Organization dedicated to promoting sustainable development
_____ Humanitarian help to children in Africa
_____ Organization dedicated to mammary cancer research
_____ Help to abandoned dogs
_____ Medical help to men suffering from prostate cancer

Figure 1. Charity descriptions used for preference survey. Participants ranked their charity preference from 1 to 6, with 1 being the highest (most likely to donate) score.

profile of the charitable target group plays an important role in determining the donor's charity preferences. The more donors are alike to something or someone, the more likely they are to help them. For this reason, donors tend to choose to help charities that help those with similar backgrounds to themselves. Our experiment tested this hypothesis, and we concluded that similarity does in fact play an important role in charitable giving decisions.

To test our hypothesis, we developed a survey which collected personal demographic data from subject participants. The survey then asked the participants to rank six different causes in order of preference. The survey was formulated to detect any favoritism to causes associated with the same race, species, and gender as the participant. Of the six possible answers, two charities compared race preferences, two charities compared gender preferences, one charity was an animal-related charity, and the last charity was a random choice not associated with any of the

previous charities. To assess the impact of the race variable, we analyzed the preference of the two race charities by the subject. If the subject gave a higher rating to the same race charity, then we considered it to be a positive result, and if the subject preferred a different race charity, we deemed it a negative result. To test the impact of gender, we carried out the same process of comparison with respect to the two gender-based charities. Finally, to test the preference for an animal-related charity, we identified the average position selected with respect to the other five "human"-oriented charities.

RESULTS
Race Test

We asked a group of people to rank six different charities in order of preference, with 1 being the highest score (most likely to donate to) (**Figure 1**) . Two of those charities were race-related. Of these two, one charity helped poor African children and the other helped poor Spanish children. The other four charities were of a different nature, serving as fillers with respect to the race test, so that the subjects were unaware of our race analysis. For the race test, we only paid attention to the relative ranking of just the two race-related charities. A subject that ranked the race-related charity identified with his/her own race profile higher than the race-related charity that differed from his/her race profile was given a positive result, and a subject that ranked the race-related charity that differed from his/her race profile lower than the race-related charity
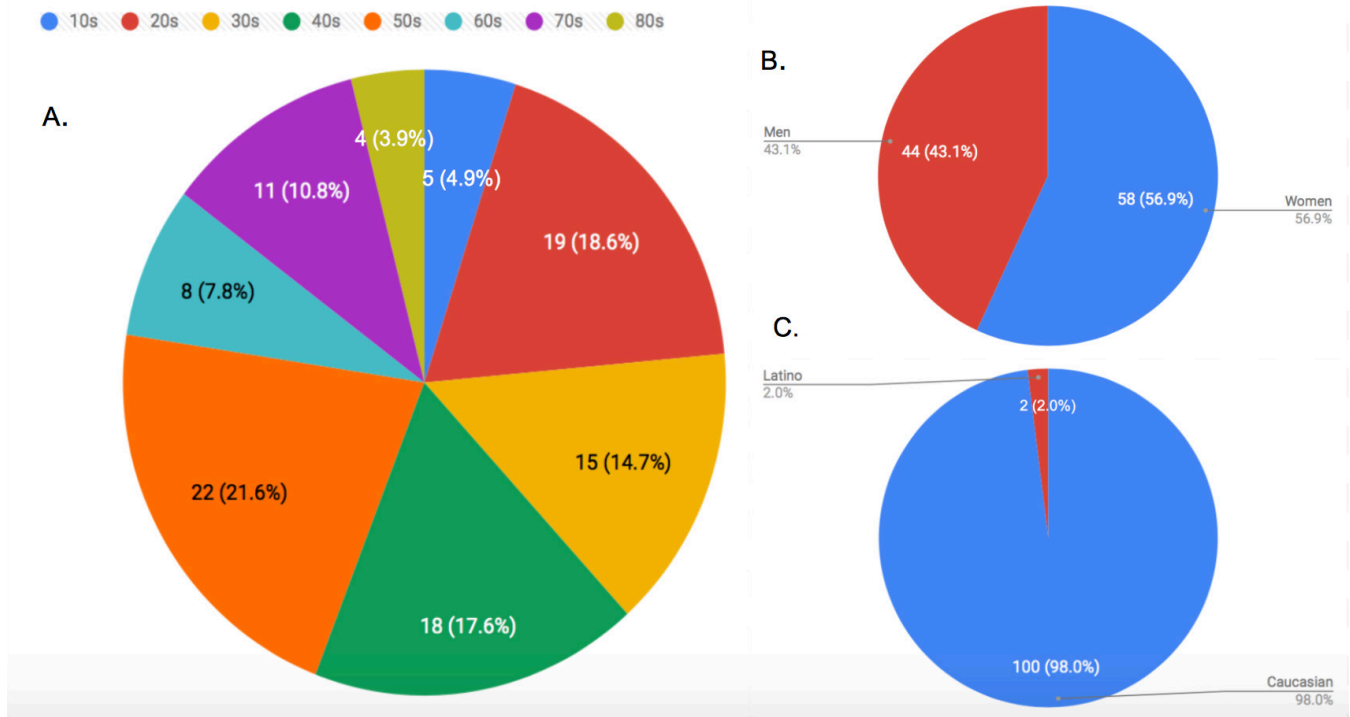

Figure 2 . Distribution of age, sex, and race of survey participants. (**A**) A pie chart illustrating the distribution of ages among the participants. (**B**) A pie chart illustrating the distribution of women and men among the participants. (**C**) A pie chart illustrating the races present among the participants.
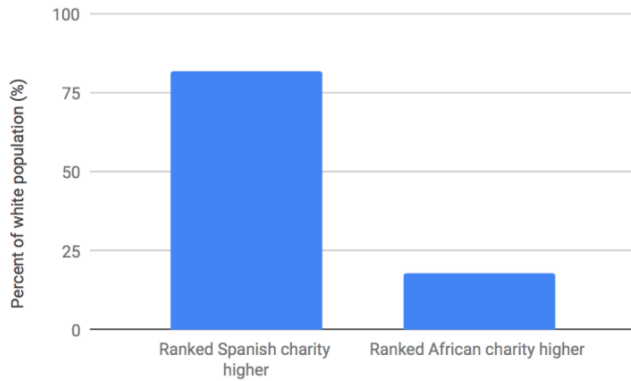
Figure 3. Graphical results for the race test. Graph illustrating what percentage of the Caucasian population favored the Spanish charity and the African charity. The graph shows how the Caucasian population preferred the Spanish charity.
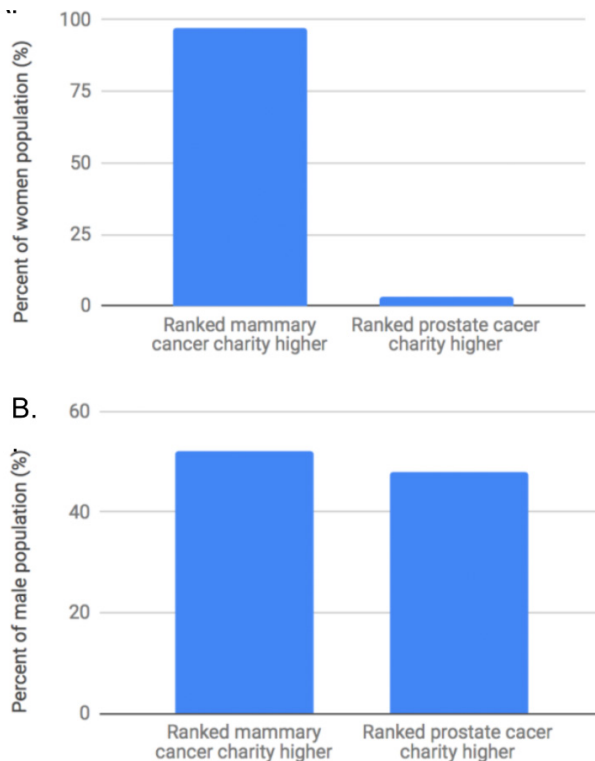

Figure 4. Graphical results for the gender test. (**A**) A graph illustrating what percentage of the female population favored the mammary cancer charity and the prostate cancer charity. (**B**) A graph illustrating what percentage of the male population favored the mammary cancer charity and the prostate cancer charity.

supporting similar traits, the white Spanish children charity (**Figure 3**). Those who preferred the Spanish children charity ranked it by an average of 1.98 choices higher than the African children charity within the 1-6 score scale (*t*-test, *p*-value = 0.032).

Gender Test

For the gender preference test, we defined two of the six survey charities as gender-oriented charities. The charities
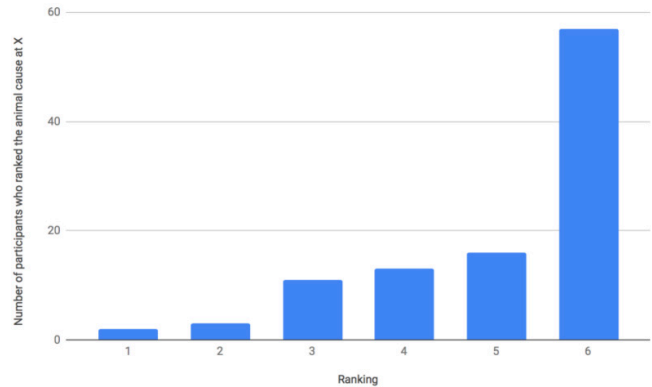

Figure 5. Graphical results for the species test. Graph illustrating the number of subjects that ranked the animal cause at each level. Note that 1 is the highest ranking and 6 is the lowest ranking. The graph shows how the majority of people gave the animal charity fairly low scores.

tested were mammary cancer as a female-oriented charity and prostate cancer as a male-oriented charity. If the subjects showed preference for the charity that shared similar gender traits (by ranking this cause higher up), then the survey result was a positive with a numerical value 1. If the subject selected preference for the charity with a different gender trait (by ranking this causes lower than the other), then the survey results for that participant was deemed negative with a numerical value 0.

The overall survey resulted in 77% of the subjects preferring the charity supporting people with similar gender traits. On average, those who preferred the cancer charity related to their gender ranked it by an average of 1.66 choices higher than the selection for the cancer charity related to the opposite gender.

23% of the participants ranked the cancer charity related to the opposite gender above the cancer charity related to their own gender. Within the female subgroup of 58 subjects, 97% preferred the mammary cancer cause above the prostate cancer cause (**Figure 4**), and on average, selected mammary cancer by 1.68 degrees above prostate cancer. However, the 44 male subjects also preferred the mammary cancer charity (**Figure 4**). 52% ranked the mammary cancer higher by an average of 1.62 degrees.

Animal Test

Of the charities we asked the population to rank, there was just one related to animals. The relative positioning of this charity to the other human related charities tells us about preference for animal-related charities. The majority of the sample population favored human-related causes rather than animal-related causes. The average placement for the animal cause was position 5.04 on the 1-6 scale. Only two subjects ranked the animal cause as the most important, while 57 ranked it as the least important (**Figure 5**). In total, 64.7% of the subjects surveyed selected the animal-related cause behind all four human-related causes, 12.7% of the subjects

selected the animal-related cause behind three of the four human-related causes, 12.7% of the subjects selected the animal-related cause behind two of the four human-related causes, 7.8% of the subjects selected the animal-related cause behind one of the four human-related causes, and only 2.0% of the subjects selected the animal-related cause before all four of the human-related causes (**Figure 5**).

DISCUSSION

Our initial hypothesis was that individuals are biased towards supporting groups or causes that are similar to themselves in terms of race, gender, species, etc. If this hypothesis is true, it could explain why animal-focused organizations have a harder time fundraising than organizations focused on helping humans. It would also have implications on how NFPOs should fundraise, define target sponsor segments, and efficiently use their investments in fundraising.

With regards to the race test, the fact that almost all Spaniards prefer helping the Spanish kids shows like-race favoritism was present, supporting the hypothesis. That the p-value was smaller than 0.05 indicates that there is a statistically significant difference between the preference for the Spanish children charity and the African children charity. This means that the like-race favoritism was strong, further supporting our hypothesis.

It is important to note, however, that the reason for such results might not be due to the theory that we support those most similar to ourselves. The Spanish charity might have been picked because it was generally more appealing. While the African and Spanish charities were presented in the most similar way possible, there could be other aspects rather than race that could have caused a favoritism towards the Spanish charity. Ideally, both Spanish and African people would have been tested for this experiment. To really support the hypothesis, the results should have shown how a group of Spanish participants chose the Spanish children charity in a significantly higher proportion, and a group of African participants, for example, chose the African children charity in a significantly higher proportion.

Another reason why Spaniards preferred this charity might not be solely because the children were of their own race. In fact, this test did not only test race but also nationality and geographic location as the two charities compared differed in all three ways. The results, thus, show that it is not just that we prefer helping those of the same race, we also prefer helping those near us nationally and geographically. The reason this might be, however, can vary. One hypothesis is that the pure fact that the children share the person's race, nationality, and geographic location pushes them to help those children. However, other factors can be at play. For example, some Spaniards might prefer to give money to Spanish organizations so that the Spanish economy (the economy that affects them most directly) becomes richer. Also, they might trust Spanish-based NFPOs more than African-based NFPOs. Thus, they

would choose to help the Spanish kids not because they are Spanish, but because they trust more that the Spanish association will actually use their money for a good cause.

With regards to the gender test, the female subjects' preferences suggested that humans prefer helping those like themselves, in this case those of the same gender. The results for the male subjects, however, supported the null hypothesis that preference is not tied to gender. This may be because, while in the race test the choices were very clear and easy to understand, the choices in the gender test may not be equal.

That is, the level of awareness of mammary cancer versus prostate cancer is not the same. The level of awareness of mammary cancer is higher than that of prostate cancer (7). Hence in the gender experiment, two forces influenced the results – the similarity bias and the level of awareness of the two cancers by the subjects.

Overall, however, we believe these results show that some form of gender favoritism exists. However, other forces could also have been at play. For example, women might have chosen to support mammary cancer not because it is a cancer that affects women, but because it may be a cancer that affects more people in general. Also, more women might have supported mammary cancer because it is a cancer that affects them individually not because it is a cancer that affects a certain gender in most cases. If some of the women surveyed suffered from mammary cancer, it is very likely that they choose this cause really to help themselves. While it is unlikely that all women who answered the survey suffered from mammary cancer, it is true that some of the women might have. While this might also explain why some men chose prostate cancer, it is important to consider that prostate cancer is less common, so the likelihood that this is the case is smaller. Nonetheless, the fact that women might have chosen the mammary cause and men might have chosen the prostate cause because they themselves were suffering from the disease reduces support for the hypothesis.

Finally, for the animal test, the subjects' overwhelming preference for human-related causes versus animal-related causes further supports the hypothesis that we are more likely to help those like us. These results further explain why it is so difficult for animal causes to compete for charity funds – the recipients of the charity are the most dissimilar from the population of donors. However, the reason for such results might not have been simply because of like-species favoritism. Other forces could have been at play. For example, people may prefer the human-oriented charities because those charities are better known.

Another factor that could explain the preference for human charities over animal charities could be an idea that the human species has a higher level of self-awareness and hence its suffering is greater than that of animals. These concepts could be ingrained in religious beliefs that place the human as a special species.

For all three tests, it is important to consider the role wording might have played in the results. Certain charities

were presented in the survey with a different writing style. For example, the description "organization dedicated to helping poor children in Spain" is written more actively than the other choice, "helping children in Africa". Such differences could have been at play, pushing people to pick the option that was better written. This would be a serious flaw in the experiment as wording would be involuntarily pushing people to certain option, disguising what they actually prefer. However, most charities were presented in a somewhat similar manner, so we do not think this flaw is serious enough to make the experiment inconclusive. While alternative explanations for the results exist for each test, we believe that the results in general provided substantial evidence that supported the hypothesis: people prefer to help those like themselves.

As the sample of subjects surveyed was quite large and varied, it is important to be cautious when generalizing the results to the entire population. This is especially true when considering that the sample consisted largely of white Spaniards from a specific neighborhood in Madrid.

For NFPOs, these are important findings to consider. With this information, NFPOs can better understand where their prospective support will come from and thus they can implement more effective fundraising strategies to reach specific target segments that is most likely to donate to their causes.

At first glance the results may seem discouraging for organizations aimed at helping those different from the general population. However, the results do show that for those causes there is a small sample of the population willing to help the cause. With this in mind, NFPOs understand how important it is to create specifically aimed fundraising campaigns. It is not efficient to carry out general fundraising campaigns to the whole population when only a very small portion of the audience will be moved to donate. What NFPOs need to do is find a way to target that small group, wisely using their fundraising resources. For example, our research showed that 2% of the participants ranked the animal cause first. While this might seem like a very small and thus discouraging portion, there is in fact a small proportion of the general population that breaks from the norm and supports animal causes. Animal NFPOs should perform research to find the specific profile characteristics of this small segment of the population and design their fundraising activities to reach that specifically defined segment.

Furthermore, the reason why people tend to prefer helping those similar to themselves might be simply because they have more information about issues affecting people like them. For example, women are probably more aware of the harmful effects of breast cancer than prostate cancer, a cancer that will never affect them. Understanding this is crucial for NFPOs. The reason we like helping those similar to ourselves is because we better understand them and can thus empathize more. Through campaigns, NFPOs should aim at educating people about their cause so that they can become as sympathetic as those who are similar to the beneficiaries

of the cause.

Many future research questions arise from this investigation. It would be interesting to isolate different age groups and nationalities to see if the importance of similarity is different for distinct generations and nationalities. Also, as this investigation underscores the necessity of profiling, it would be important to further explore the most effective ways of doing this.

MATERIALS AND METHODS

To test the hypothesis, we created a survey which was completed by all participants. The survey consisted of two sections. The first section asked for the participant's age, race, and gender. The second section asked the subjects to rank six causes in order of preference. To quote, the survey instructions read, "Please rank the following causes from 1 to 6, 1 being the one you are most interested in actively helping". For each of the causes, we did not use names of charities but instead described the cause. In **Figure 1**, the descriptions we used for each charity are shown. After extensive surveying of random people on the streets, information from 102 subjects was collected.

The experiment was conducted at Plaza de Felipe II in Madrid, Spain. Plaza de Felipe II is located in a middle to upper middle-class neighborhood of Madrid. It is a highly transited commercial area of Madrid. The surveyor asked the passersby if they would be willing to take a survey regarding their charitable organization preferences. They were asked to sign a consent form and then completed the survey. The surveys were performed over a period of four evenings from 6-8 pm. Those that took the survey were offered a paper survey and a pen to mark their preferences.

To analyze the relationship between the subjects' race, gender, and species and their preferences we observed the relative positioning of certain charities. In the gender and race test, we identified if the charity of the participants' same gender/race was preferred over the charity of unlike race/gender. This was accomplished by comparing the rankings (1-6) of each of the two charities (the two race-related charities for the race test and the two gender-related charities for the gender test). If the participant placed the like-charity at a higher ranking, the response resulted in a positive 1, while a lower ranking of the like-charity counted as a 0. For the species test, we calculated what percentage of the subjects placed the animal-related charity in each rank position.

Of the six charities presented, one was a "filler" charity concerning environmental causes. This cause was included as a filler choice to distract participants in order to make it difficult, if not impossible for participants to understand that the survey was testing the relative race and gender preferences. If this cause was removed, it would be more evident that some causes were grouped in pairs and were only different with regard to gender or race. This could have placed pressure on the participant and biased their responses.

When analyzing, we also carried out a *t*-test for the

race test results. Microsoft Excel was used to calculate the percentages and averages and to perform the statistical calculations and the *t*-test.

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