

SILVER HUMAN PRACTICES & GOLD HUMAN PRACTICES

MAKING SCIENCE SERVE THE PEOPLE

ONLINE RESEARCH

Written By Yuchen Tian

Noticing that there is an increasing gap between the supply and demand of blood for transfusion around the globe, our group drafted a project that aims to create hemoglobin based oxygen carriers. Before starting the experiments, we did some research on this topic to gather information. Here are some key information we found.

WE NEED BLOOD

In a car accident, one victim can require as many as 100 pints of blood.

For every 7 people entering a hospital, about 1 of them needs blood.

Patients with severe sickle cell anemia need to receive blood transfusions every month to survive.

Every 2 seconds, someone in the United States needs a blood transfusion to save his life.

WE ARE SHORT OF BLOOD

Among the 500000 women who die each year during pregnancy and childbirth, haemorrhage, a condition that invariably requires blood transfusion, is the most common cause.

Although 38 percent of the United States population is eligible to donate blood, less than 10 per cent of that group actually does.

CURRENT BLOOD DONATION IS UNSAFE

Globally, up to 4 million people have been infected with HIV by the transfusion of unsafe blood.

The prevalence of hepatitis B, hepatitis C and syphilis in donated blood is still extremely high in many developing countries; the prevalence of Chagas disease in donated blood is a major problem in South and Central American countries.

ABOUT ARTIFICIAL BLOOD

Artificial blood is a product made to act as a substitute for red blood cells.

Red blood cells substitutes or synthetic oxygen transporters studied so far are perfluorocarbon, also known as PFC, and Hb based substitutes.

After over 100 years of research, there still have not been any successful artificial red blood cells currently in use.

Short circulation in human body, immune response, and increased probability in stroke are all current problems for artificial red blood cells.

After seeing these statistics, we steeled our resolve to do this project. Our goal was to engineer human cell line to produce exosomes that work as cellular hemoglobin based oxygen carriers, which can be used in blood transfusion and stroke treatment.

CITATION

[http://www.who.int/worldblooddonorday/campaignkit/
WBDD_GlobalNeed_English.pdf](http://www.who.int/worldblooddonorday/campaignkit/WBDD_GlobalNeed_English.pdf)

<http://fourhearts.org/facts/>

<https://www.healthline.com/health-news/blood-donations-at-critically-low-levels#2>

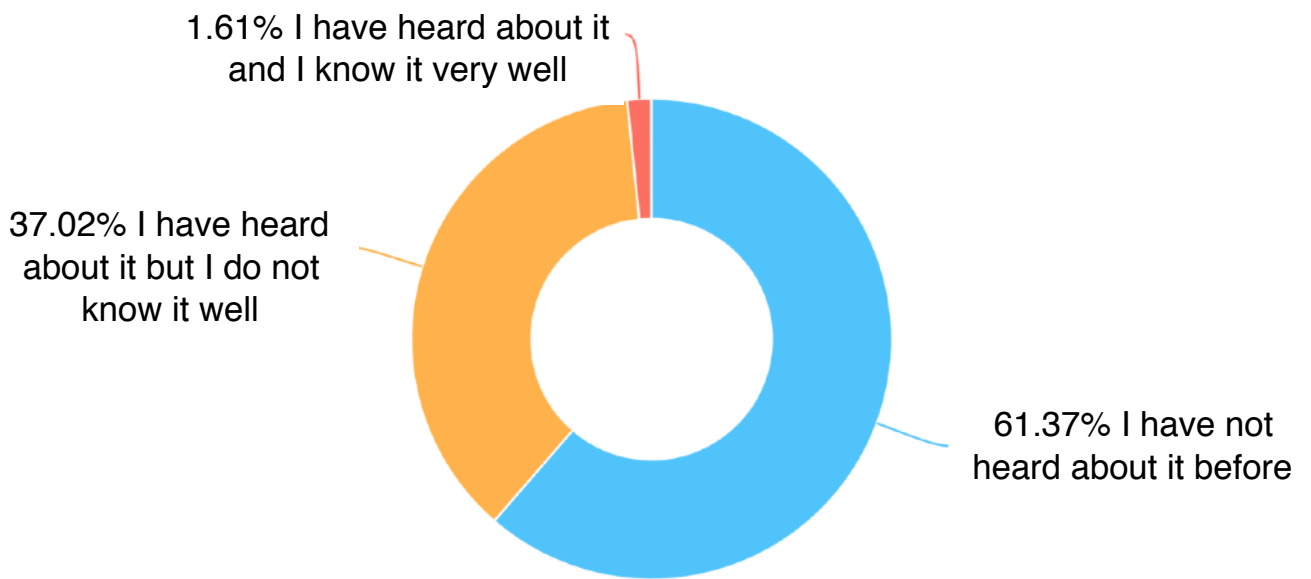
PUBLIC OPINION SURVEY

Written By Yuchen Tian

In order to understand how the general public view artificial blood, we created a survey to gather opinions from different groups of people. Over 500 people filled in our survey; the results are shown below.

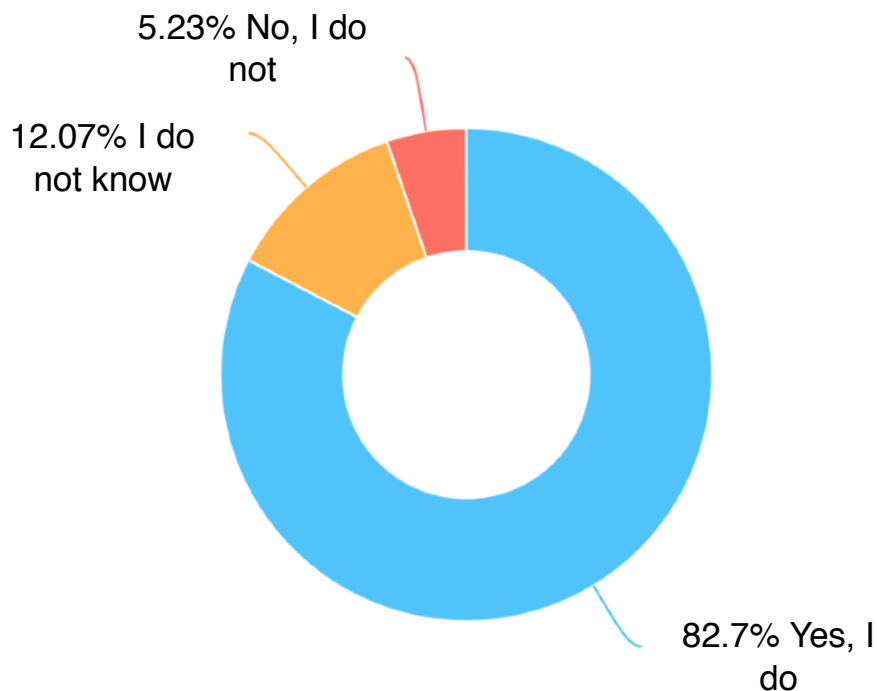
Q1

Have you ever heard about artificial blood before?



Q2

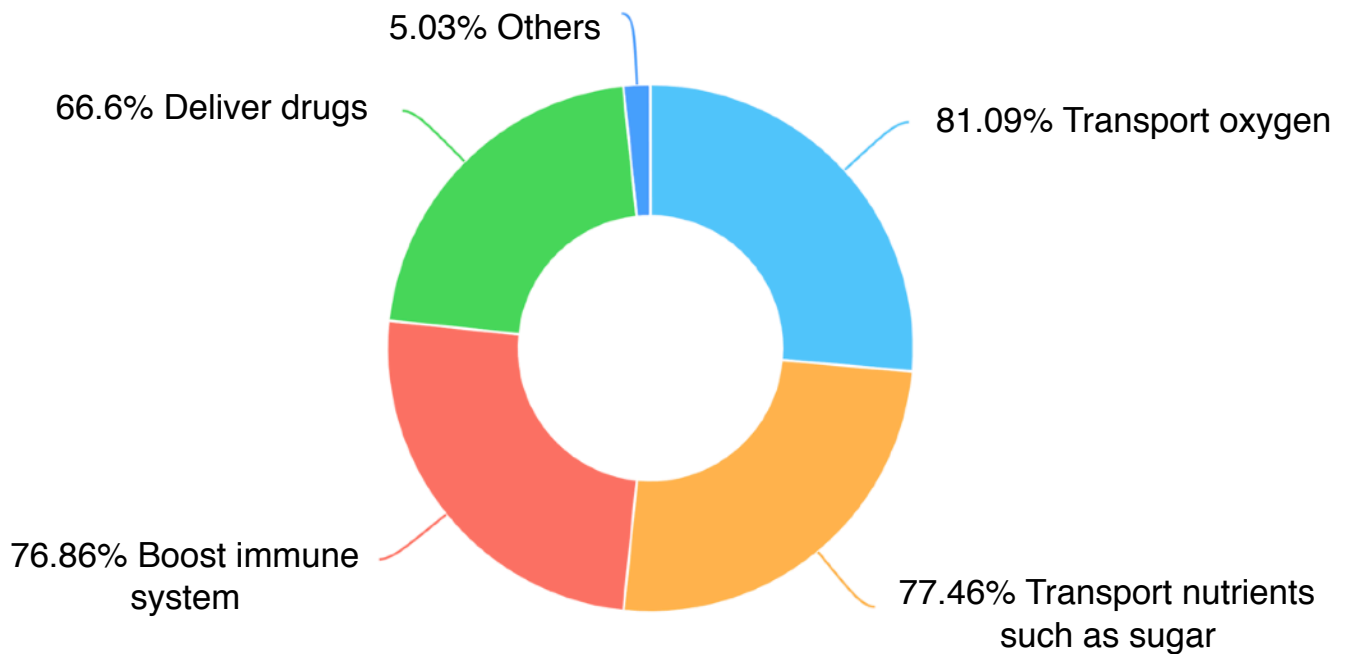
Do you support the development of artificial blood that can replace part of the blood function?



Q3

In your opinion, what functions should artificial blood have?

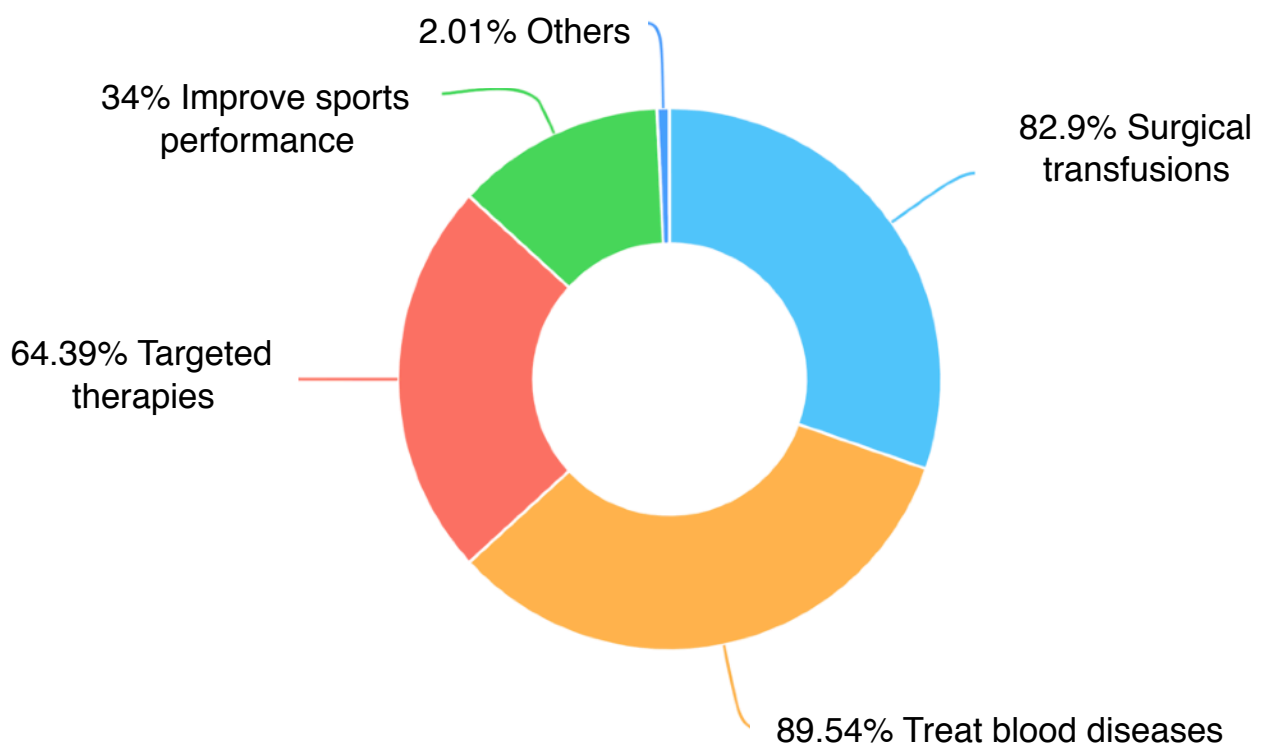
[You can choose more than one answer]



Q4

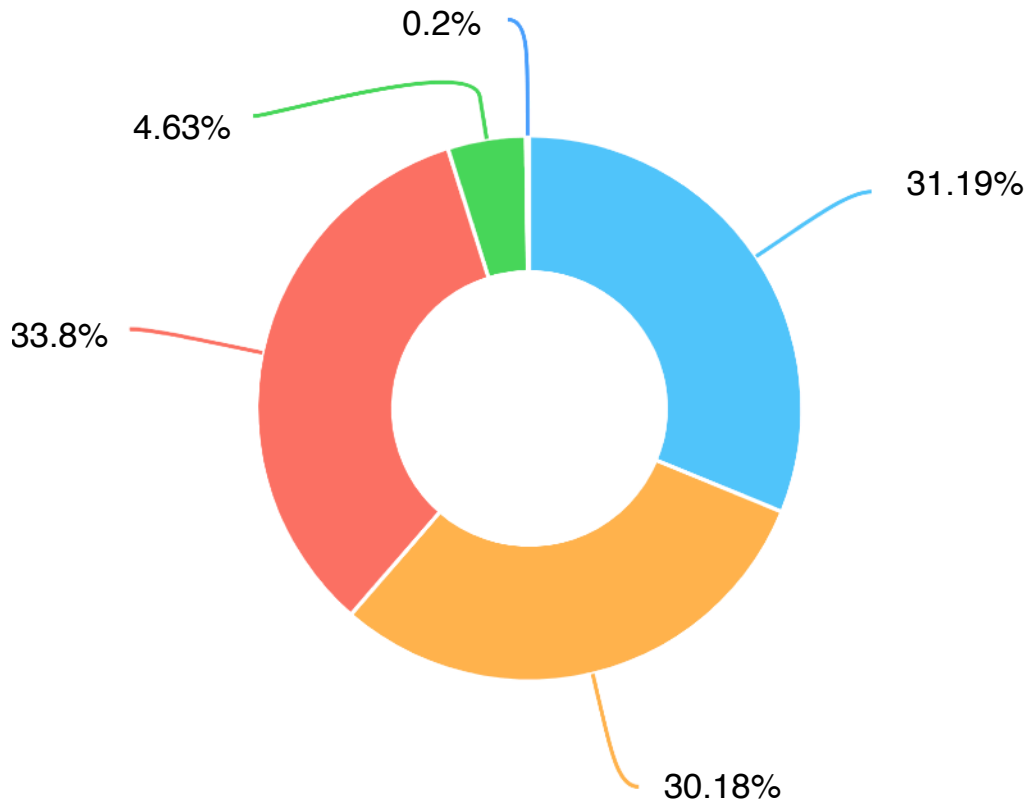
In your opinion, what could artificial blood be used for?

[You can choose more than one answer]



Q5

In your opinion, in which areas can we use artificial blood if it is available?
[You can choose more than one answer]

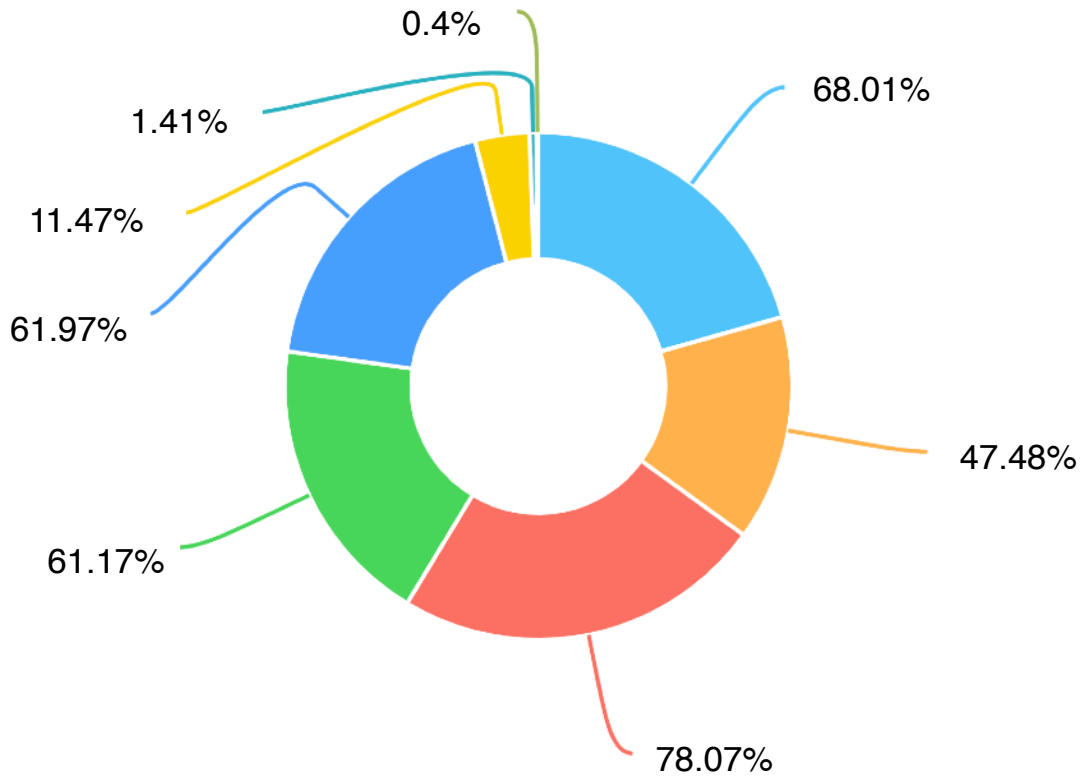


- For both medical and health care purposes
- Only for medical purposes, such as blood transfusions
- Only for specific diseases such as inherited blood diseases
- Only for scientific research in small scales
- Should not be used under any circumstances

Q6

Currently there are some problems in human blood transfusion, which of the following problems do you think are very serious?

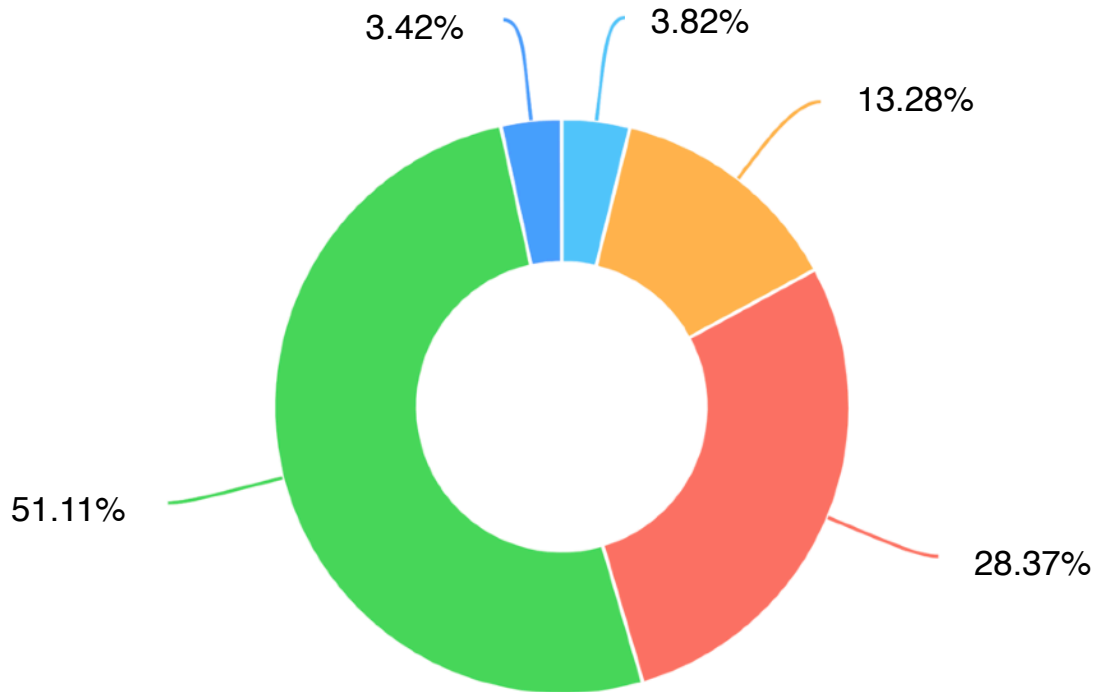
[You can choose more than one answer]








- The only sources of human blood are people who volunteer to donate blood
- High costs for testing, transportation, and storage
- Transmission of viruses such as HIV
- Limited supply of blood, especially for rare blood types
- Complications such as hemolysis, fever, and allergic reactions
- Ethical issues
- None of the above
- Others

Q7

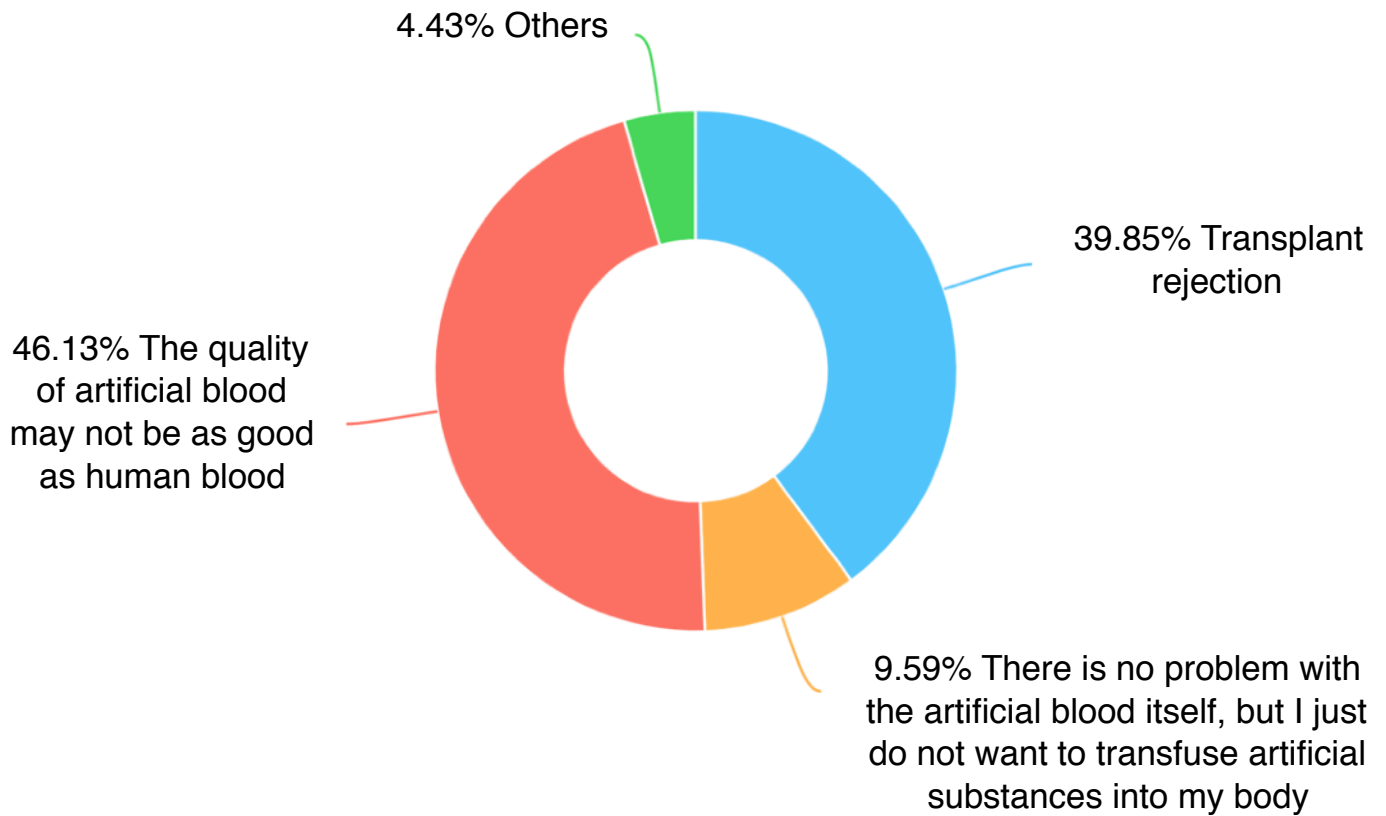
If you are to accept a blood transfusion, would you prefer natural blood or artificial blood?



-  I can only accept artificial blood
-  I prefer artificial blood over human blood
-  I have no preference
-  I prefer human blood under normal circumstances
-  I can only accept artificial blood

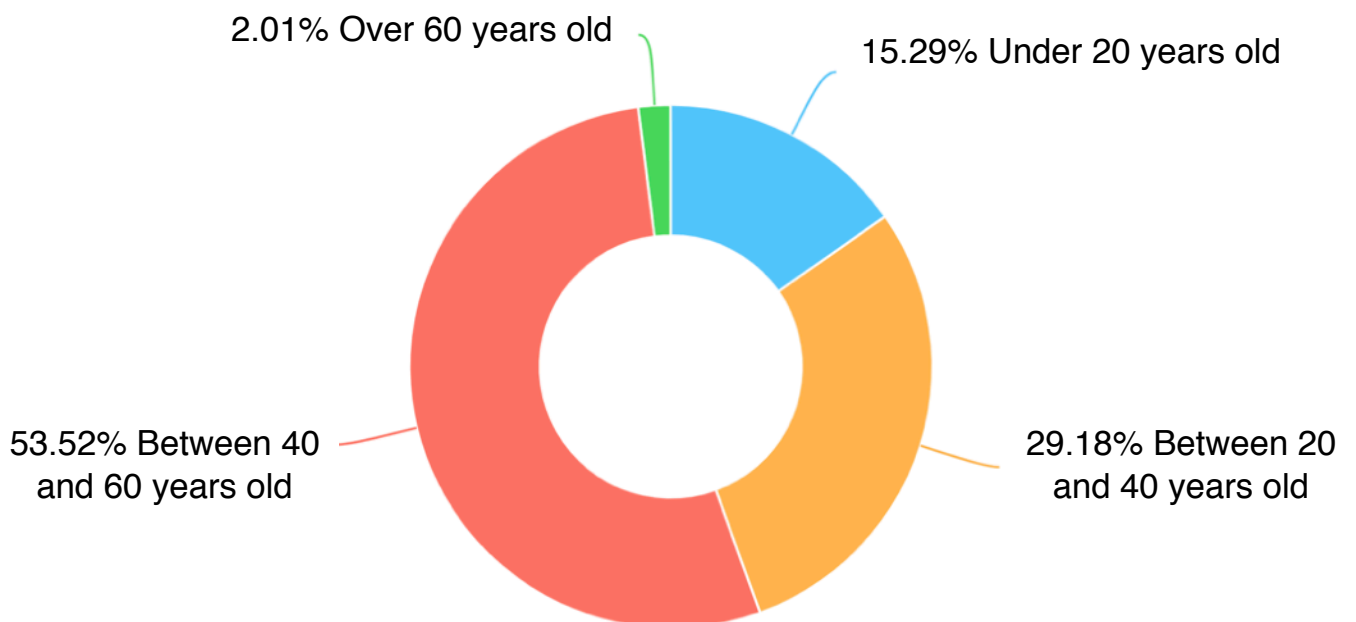
Q8

If you prefer human blood, which of the following problem about artificial blood do you worry the most?



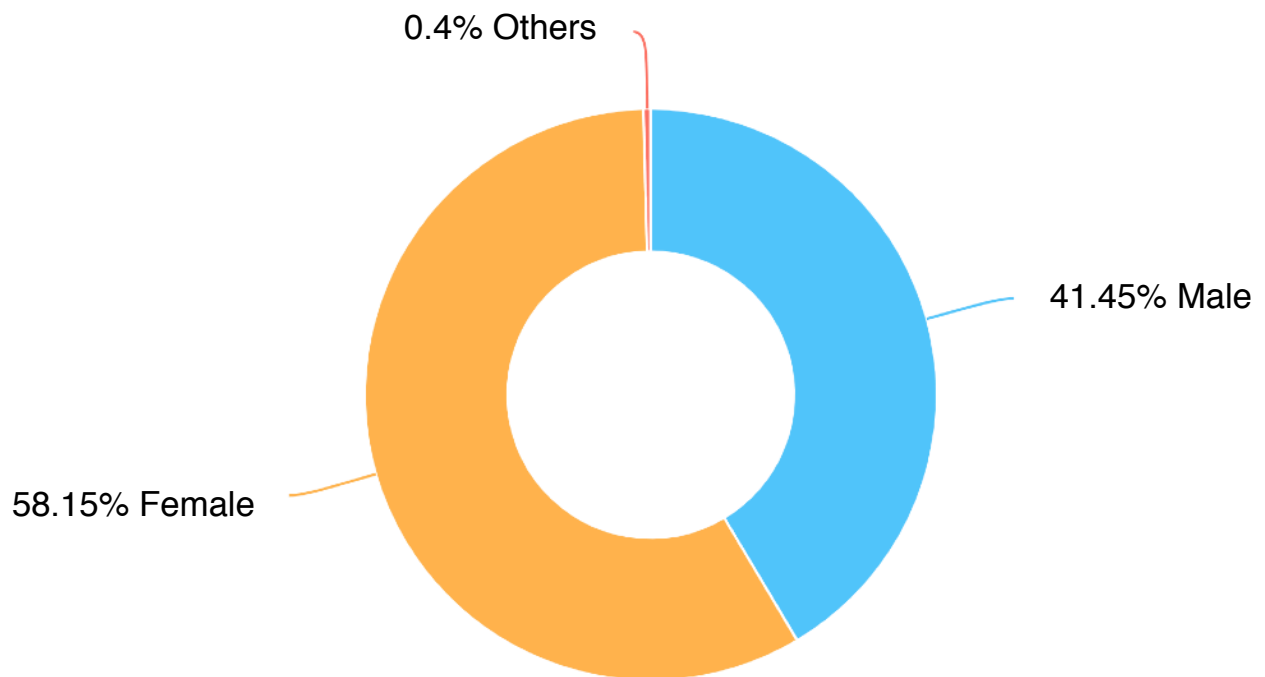
Q9

How old are you?



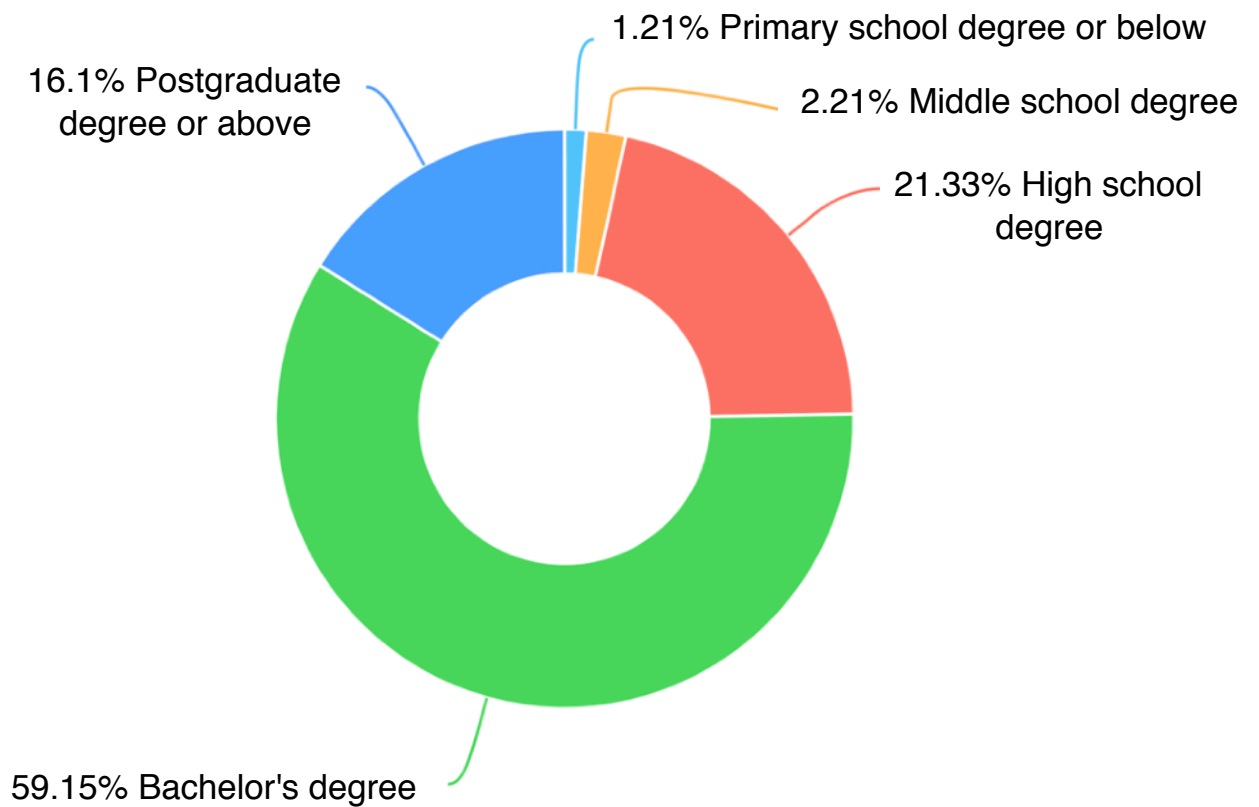
Q10

What is your gender?



Q11

What is your level of education?



RESULT ANALYSIS

Q1

Most people have never heard of artificial blood, so it is our responsibility to introduce it to the general public.

Q2

The majority of people support the development of artificial blood, which means that our project is supported by most people.

Q3

People think artificial blood should not only have the same functions that natural blood has, but also should be able to help people fight diseases.

Q4

Most people believe that artificial blood can be used for medical purposes, and about one third of people believe that it can be used to improve athletic performance.

Q5

Most people believe that artificial blood can be used for medical purposes, and about one third of people believe that it can be used to improve athletic performance.

Q6

People are most concerned about the safety of natural blood; they also think the limited supply of natural blood is a problem. If we develop artificial blood, we would aim to solve these problems.

Q7

Currently, the majority of people more readily accept natural blood than artificial blood, or have no preference between the two. We aim to help people relieve their mental barriers, so that more of them can accept artificial blood.

Q8

People who prefer natural blood are mainly concerned about the quality and safety of artificial blood. We can address these problems in our presentations to tackle their concerns.

Q10 to Q12

We created these three questions to ensure that people who did this survey can represent the general public.

In our opinion, raising public knowledge on blood transfusion is crucial to the development of artificial blood. We believe the power of education and engagement at social and personal levels can help us achieve this goal.

SYNTHETIC BIOLOGY DATABASE

Written By Sihan Yang

“How can we select a meaningful and practical subject for the project” is always a good question to think about in the synthetic biology research. For iGEMers, especially for high school students, we are struggled at first to choose our own projects like many others. There is only one clue, that is, we can refer to the database of previous iGEM teams. However, all those previous iGEM projects are also started by some freshman and they are just some preliminary researches comparing with those successful projects which have already evolved into large synthetic biological startups or even IPO listed companies. Numerous researches failed due to the project design in the initial stage where a large amount of capital could be saved by just reading some more successful projects and careful design. Instead of only reading the previous preliminary iGEM teams' projects or posters, we want to dive into some more successful projects that are more mature, including those projects that have already changed the world. Unfortunately, there is not such a database that could be understood by the freshmen.

Still, there are nearly nine hundred synthetic biology related companies that have overcome the difficulties and successfully become listed on NASDAQ and some more in other stock markets. There are even more projects that have come out of the lab and got invested by the venture capitals. As to a freshman intruding the biological field to start any research, these projects are the good examples to study. Studying enough successful cases could be helpful to find out how a successful project is started and evolved. Most importantly, it is the one of the best ways to learn how to evaluate the feasibility of a project in the early stages. But where can we find them?

Based on this idea, our team act to formulate a small database of some more successful synthetic biology projects as one of the human practice work so that we could learn some more cases during the preparation and share it with all the coming teams. We hope it could evolve into a wikipedia of synthetic biology and iGEM case study resources. It is basically composed of two types of companies: one type is some famous biological companies that have already become listed in the open stock market. The other type is some emerging startup synthetic biological companies who were already invested by the venture capital investors .

These projects are all outstanding in the relevant specific areas of study and could be the inspiration for newcomers who have just ventured into the biological field. Imagining that you standing at the top of the pyramid, you will definitely get a whole picture of the industry. There are several basic categories of company information included: the companies' history, including the abstract, purpose, development of main products and approaches of the company. The information is highly summarized and arranged in an order which is conveniently accessible for everyone. It is also fantastic pieces of approaches to obtain more knowledge of research and development collaboration between companies. On the other side, by analysis of the emerging companies, everyone will have a quick glimpse of the most popular topics of the current time to foresee industry trends. Take one company for example that was described in our database, Intellia which is a biotechnology company developing biopharmaceuticals using a CRISPR gene editing system. We display the milestone along the history to see if there are some patterns. From the skyrocket growing transfers to the stable stock price which IPO had successfully closed with the full 6.9 million shares sold, Intellia is an indisputably fast booming genetic engineering company .

Due to the limitation of time, we only include a small number of companies on the list. However, it is an initiation project and attempt which has a colossal potential. From this point, we can make more innovations. It can be adapted to a unique platform with vitality. People could keep devoting more information to extend the database which makes the collection of larger diversity. We hoped that it would help more people including ourselves in the future.

For the full version of our database, please visit http://parts.igem.org/wiki/images/a/a0/T--SHSU_China--Companylist.xlsx.

Program Info				
Year	Business	Progress	Partner	Results
		Project		
2014	license gene-editing technology from Caribou and contract with the company for research and development services.		Caribou	
2014	Series A investment round that raised \$15 million in cash.		Atlas Venture and Novartis	
2015	Novart and Intellia announced a five-year research and development collaboration focusing on the use of CRISPR-Cas9 in developing chimeric antigen receptor T		Novartis	

	A	B	C	D	E	F
1	Name	Name URL	Type	Descript	CB Rank	
2	Moderna	https://	Organiza	Moderna	1,862	
3	Caribou	https://	Organiza	Caribou	3,016	
4	Synlogic	https://	Organiza	Synlogic	6,405	
5	Poseida	https://	Organiza	Poseida	7,090	
6	Vedanta	https://	Organiza	Vedanta	7,917	
7	Cell Des	https://	Organiza	Cell Des	8,817	



Moderna Therapeutics

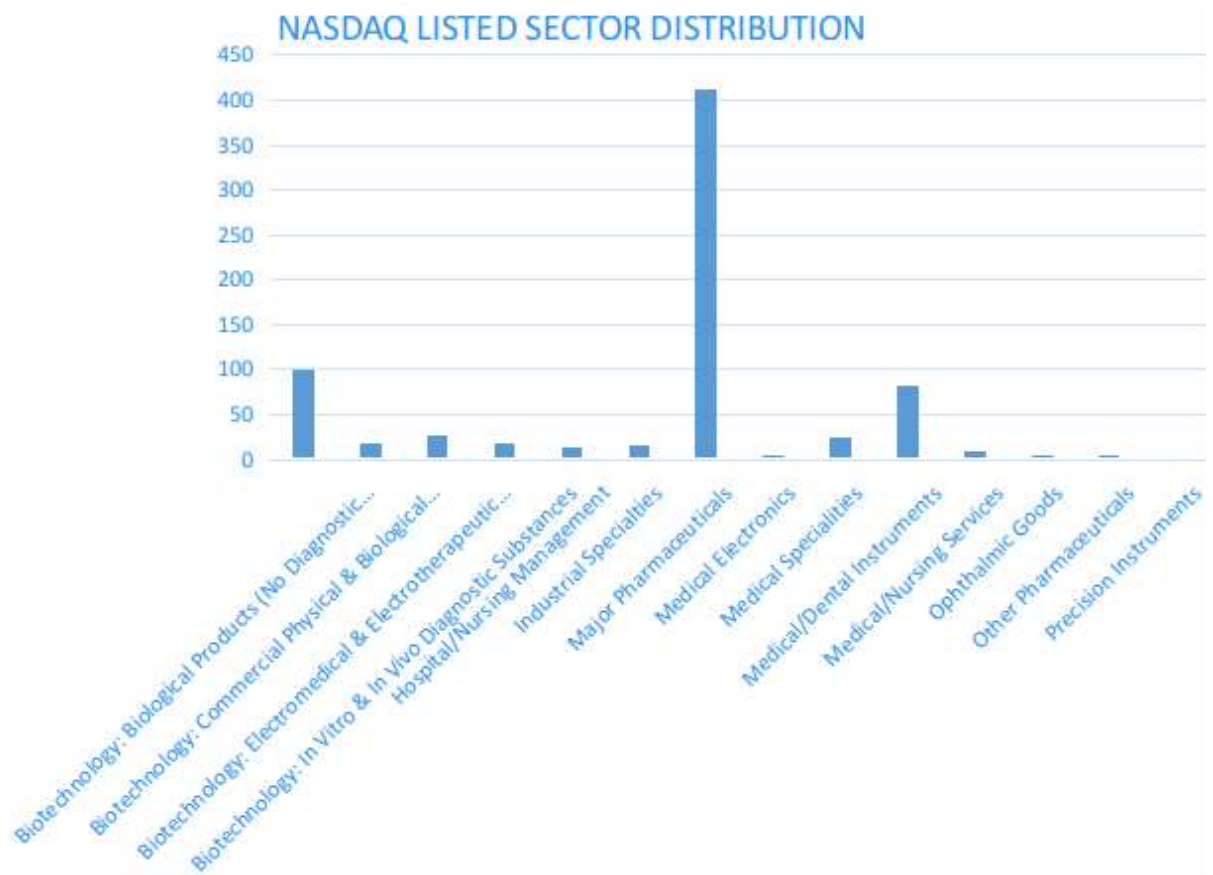
Moderna Therapeutics specializes in drug discovery and drug development based on messenger RNA.

[Cambridge, Massachusetts, United States](#)

Categories	Biotechnology , Genetics , Health Care , Medical , Pharmaceutical
Headquarters Regions	Greater Boston Area , East Coast , New England
Founded Date	2010
Founders	Noubar Afeyan , Robert Langer , Stephane Bancel
Operating Status	Active
Funding Status	Late Stage Venture
Last Funding Type	Series H
Number of Employees	101-250
Also Known As	Moderna
Hub Tags	Unicorn

IPO Status	Private
Company Type	For Profit

Website	www.modernatx.com
Facebook	View on Facebook
LinkedIn	View on LinkedIn
Twitter	View on Twitter
Contact Email	info@modernatx.com
Phone Number	6175485422



EXPERT INTERVIEWS

Written By Yuchen Tian

In order to get more insight into the topic of artificial blood, our group member Yuchen Tian interviewed six experts face to face. These professionals are all involved in fields related with artificial blood, from the head of hematology department to the investor of various biomedical companies.

INTERVIEW WITH PROFESSOR ZHAI

Zhai Yonggong

翟永功教授

Professor at the School of Life Sciences at Beijing Normal University

Visiting scholar at the School of Pharmacy at University of Pittsburgh

Expert in the field of molecular and cellular pharmacology

“Blood donation is voluntary in China, so the demand for human blood is far greater than the supply. This is why we need artificial blood, and I am glad to hear that your group is working on it.”

“But you should know that making artificial blood is very difficult. Around a decade ago, some top experts began to work on creating artificial blood cells, and they are still working on it now. In the field of medicine, it is impossible to create something new in a year or two. It can actually take up to a decade or two.”

“It is easier to create a substance that carries oxygen than to create a complete red blood cell, but it can still be hard. Instead of working on increasing its half life, I think you should first focus on minimizing the immunoreaction that your substance can cause. In order to so this, you can choose to use human hemoglobin to create the oxygen carriers. I know this can be a great challenge; but I believe you guys can do it if you have enough time. Good luck on your project.”

INTERVIEW WITH MS. GUO

Guo Jingbo

郭静波女士

Investor of various biomedical companies

Graduate student at the School of Life Sciences at Beijing Normal University

“I know a product called EPO, which promotes the ability to carry oxygen in human bodies. It is somewhat similar to the substance that you are trying to make, and I think the consumers of this kind of products are mainly athletes.”

“Scientists can already produce artificial plasma, which is used in hospitals to maintain the osmotic pressure in blood vessels. In China, scientists make artificial plasma with proteins from bovine bones, but this kind of plasma cannot be used in humans because of the impurities inside. Hence, the artificial plasma we are using comes from the United States.”

“It is true that we have artificial plasma, but we do not have artificial blood cells. Your group is trying to make something that has not been produced by any companies in the world. If you succeeded, I would definitely invest in your project. I am very happy to have young people like you to take this challenge.”

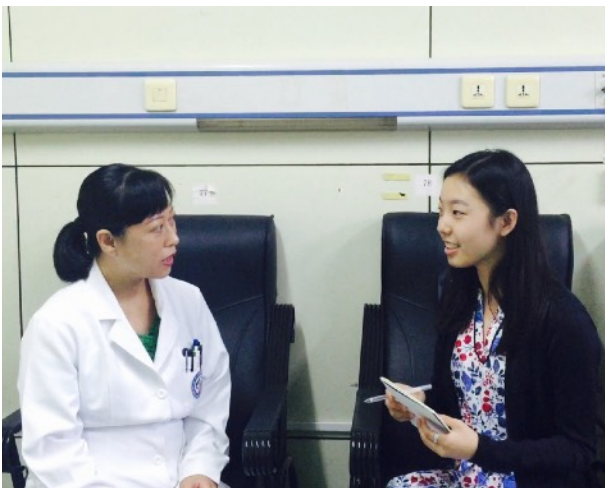
INTERVIEW WITH DOCTOR ZHANG

Zhang Dan

张丹主任

Head of the emergency department at Peking University Shougang Hospital

Graduate student at the Medical School at Peking University



“In my department, patients do not usually need blood transfusion, so if we need blood, we can get it. However, in some other departments, blood is clearly not enough. Sometimes the doctors have to postpone their surgeries simply because they do not have blood for transfusion. Every doctor in our hospital knows that he cannot use blood unless it is absolutely necessary.”

“I think blood transfusion is very safe now, but there is a small possibility that it can cause some problems. If any kind of immunoreaction happens, the patient is in danger. He might get a high fever, a syncope, or even a shock. If artificial blood can 100% prevent these problems, it will be very helpful for us.”

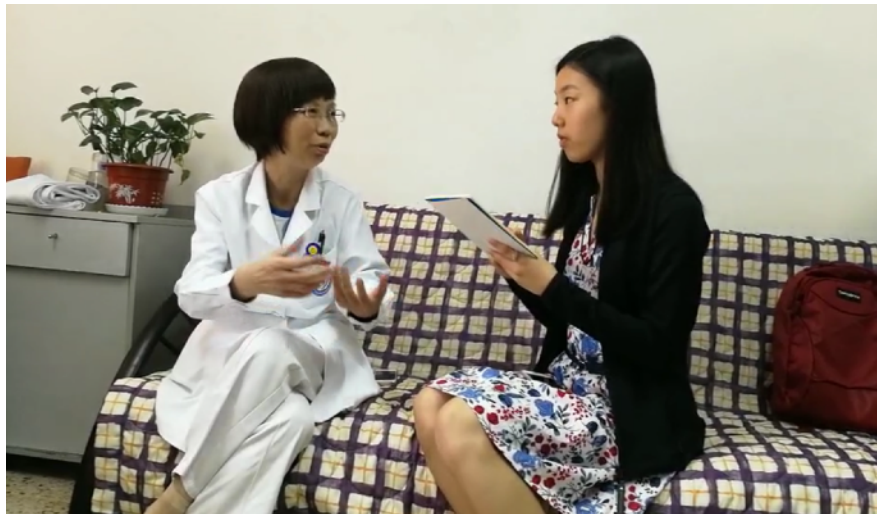
INTERVIEW WITH DOCTOR YU

Yu Yanfang

余延芳主任

Head of the hematology department at Peking University Shougang Hospital

“Hospitals are running out of blood, so we no longer transfuse whole blood into our patients unless he has a serious liver disease. In the blood bank, machines help us separate red blood cells, white blood cells, platelets, and plasma. Doctors then



transfuse different parts of blood into different patients according to their needs.”

“Blood transfusion is a lot safer now than that of before, but there are still two problems. First, we may not be able to detect some virus. In China, we check the blood for two times before it is transfused. Practitioners do a quick test before the blood donation, and a more careful inspection before the transfusion. However, some diseases such as Hepatitis B have a long incubation period, so HBV may not be detected during the inspection. HBV can infect the patient if he receives the contaminated blood. Secondly, although we cross match the blood before every blood transfusion, allergic reactions are possible. Patients might get high fevers and chills after the transfusion; they may even die. I do not have any examples for you because I have not encountered any of these situations before.”

“It is very cool that your group is trying to make artificial blood, and our hospital definitely need it. If you want to use it in hospitals, you have to make sure that it does not produce immunoreactions. Remember that immunoreactions can be fatal. Personally, I support your project. I am just a bit worried that some patients may not want to transfuse artificial blood because of ethical problems.”

INTERVIEW WITH DOCTOR QIN

Qin Xiuchuan

覃秀川主任

Head of the emergency department at Beijing Anzhen Hospital, Capital Medical University

Doctor Qin was about to preform a heart surgery when we interviewed her, so her response was brief.

“Chinese hospitals receive less blood every year. I used to have enough blood to use, but then I started to postpone surgeries because of the lack of blood. I think artificial blood can be really helpful in surgeries. It can save lives.”

INTERVIEW WITH DOCTOR SONG

Song Yandong

宋艳东医生

Doctor in the cardiology department of Beijing Anzhen Hospital, Capital Medical University



“I can tell you that hospitals really need artificial blood, but I do not think they need oxygen carriers that much. I do agree that oxygen carriers can be used to treat a small number of blood diseases, but they definitely cannot be used in surgical transfusions. Although we do not transfuse whole blood, we need to transfuse something more than just oxygen carriers. Red blood cells have a lot more functions then carrying oxygen. So if you are

trying to make oxygen carriers, you should narrow your target consumers to people with specific blood diseases and athletes. Keep in mind that this is still a large group of people. If you can manage to add more functions to your oxygen carriers, then it might be useful in surgical transfusions. Good luck on your project!”

INTEGRATED HUMAN PRACTICE IN OUR INTERVIEWS

After interviewing these experts, we created a list to summarize the common ideas expressed by them, as listed below.

- I. Hospitals are running out of blood.
- II. Hospitals need artificial blood for transfusions in surgeries.
- III. It is very difficult to create artificial blood cells.
- IV. It is important that the oxygen carriers do not generate immunoreactions.
- V. If the only function of artificial red blood cells is to carry oxygen, then the target consumers can only be people with specific blood diseases and athletes.

Interviewing the experts enabled us to see how the professionals view our research and our project about artificial blood.

Since three of the six experts mentioned that it is important to eliminate the possibility of immunoreactions, we focused on this aspect when devising our experiment. After weeks of discussions, we chose to use biogenesis to create oxygen carriers. Biogenesis can help our oxygen carriers to have long circulation time and fast production speed; it is also not as expensive as other methods. Most importantly, it addresses the major concern of the experts. Biogenesis was proven to be more immune friendly than many other methods, so we finally achieved unanimity in the usage of it.

In our experiment, we first tried to use human hemoglobin in ExoBlood because Professor Zhai mentioned this in his interview. However, we failed to get the required parts due to the complexity of this task and the time limits. Hence, we alternatively used *Vitreoscilla haemoglobin*, a kind of hemoglobin used by multiple

iGEM teams to increase bacterial survival in low oxygen environments. We subcloned *Vitreoscilla* haemoglobin from BBa_K1321200 in the iGEM distribution kit.

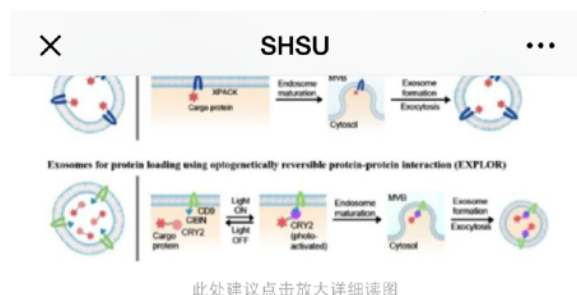
In a nutshell, interviewing these professionals helped us gain a deeper understanding of our project, so that we could better devise our experiments and educate the public.

PUBLIC OUTREACH

Written By Yuchen Tian & Yan Luo

WECHAT

WeChat is a popular social software in China; therefore our team created an official account on WeChat and published multiple articles concerning our research process and the topic itself. With the goal of fascinating the public, instead of boring them with serious scientific essays, we used our daily language, even adding a humorous tone, in discussing our research. From time to time, we referred to trending topics to make our research much easier to be comprehended and a whole lot more interesting to those who aren't involved in the scientific field. For instance, in one of articles, we introduced our research project, referring to the trending anime series Working Cells. In this way, science appears less daunting and connects more to our daily activities.



简单介绍:

其一，离体使用电穿孔等方式直接装载；

其二，在细胞内过表达该蛋白，随后便和外泌体一起分泌；

其三，通过某些膜锚定蛋白(membrane-anchored proteins)，将运输蛋白质直接结合在脂双分子层上，或是通过一个糖分子和脂双分子层连接，最后在外泌体产生时自动包被分泌；

其四，通过某种病毒复制通路进行干预。

而要做到比较实惠，推荐使用的便是第三或第四种方式，大量制造所需要的血红蛋白载氧体(Cellular Hemoglobin Based Oxygen Carrier, HBOC)。



2018iGem
高中组队伍
High School Track team
做最有趣的科研
Dedicated to Fascinate



【01 跑胶】



【02 挑菌+涂板】



TWITTER & INSTAGRAM

Since WeChat is only popular in China, we created a Twitter and Instagram accounts. Similarly, we post fun pictures and updates on a regular basis.

The image is a collage of social media content for the IGEM 2018 SHSU team. It features three main screenshots:

- Instagram Profile:** A screenshot of the Instagram profile for 'igem2018_shsu'. The profile picture is a circular logo with 'SHSU CHINA' and 'SHANGHAI UNIVERSITY OF SCIENCE AND TECHNOLOGY'. The bio reads: 'IGEM 2018 SHSU Official Instagram of IGEM 2018's SHSU Team Based in Shanghai, China igem.org'. The profile shows 9 posts, 99 followers, and 222 following. Below the bio is a grid of six photos showing team members in a laboratory setting.
- Twitter Post 1:** A screenshot of a tweet from 'IGEM 2018 SHSU @2018Shsu' dated July 10. The text says: 'We are 2018 IGEM's Shanghai, China SHSU team.' Below the text is a large image of the iGEM logo, which consists of a green gear with a cell-like structure inside, and the letters 'iGEM' in black.
- Twitter Post 2:** A screenshot of a tweet from 'IGEM 2018 SHSU @2018Shsu' dated July 10. The text says: '#WelcomeToIGEM We have received our 2018 DNA Distribution Kit 📦'. Below the text is a photo of a laboratory bench with various equipment and a white box with the iGEM logo.

On the right side of the collage, there is a partial view of a Twitter search results page, showing a search bar and a list of users with their follower counts.

EDUCATION

Last year we started the first Biobuilder synthetic biology course in Chinese mainland to introduce the novel idea of synthetic biology to high school students in SFLS. We translated part of the course materials and posters into mandarin and put them into use in our strawberry DNA extraction and colorful bacteria experiments. This has great effect on the students and on us; our iGEM team is directly formed from the original education group.



推文



翻译自英文

Tom Luo @SFLS_BioBuilder

This Christmas we have finished our very first #BioBuilder experiment. The kits are well prepared and the students all love the colorful bacteria! Thank you @SystemsSally !





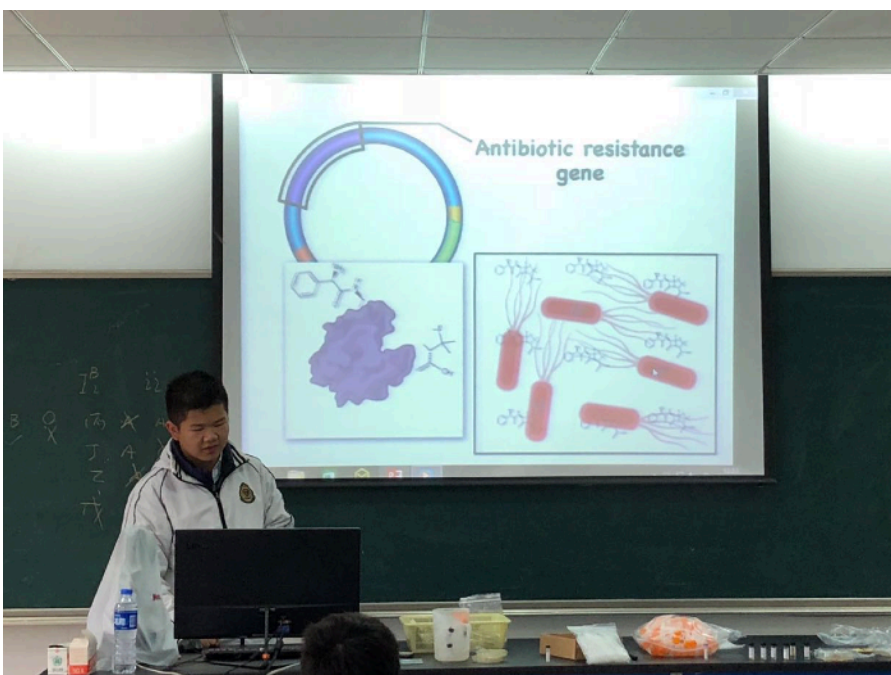
推文



Systems Sally
@SystemsSally



So excited to anticipate 2018 with [#BioBuilders](#) around the world! Thanks go to YOU [@SFLS_BioBuilder](#) for the hard work and to [@MITdeptofBE](#) [@CarolinaBio](#) + [@OReillyMedia](#) for sharing [#synbio](#) resources



ONLINE TRANSLATION

During this summer, we used our language skills to help MITx to translate its molecular biology online courses on Edx. The translation group already had 10 people and was using the transifex platform to translate the videos. We hope that through translating these courses into Chinese, we can increase their popularity, thus raising public knowledge on biology outside our school and on a rather social level.



Molecular Biology: RNA Processing and Translation

MITx - 7.28.3x
Ended - 2017年10月12日

[查看存档的课程](#)

Reading the mRNA in Translation




让我们从mRNA开始讲起
每一段mRNA在任一区域都可能
拥有3个不同的阅读框 (reading frame)
阅读框是什么意思呢?
氨基酸由3碱基一组的密码子(codon)编码
它们组成不重叠的密码
所以你就可以把这里的AUG
视作起始密码子(start codon)
下个密码不是AAA 虽说应当如此
它可以是UGA
你每次移动一个碱基
然后读接下来的3个碱基

CCIC MEETUP

Written By Yuchen Tian


During this summer vacation, our group members attended the CCIC meetup in Shanghai. During the meetup, we did a presentation about our project and exchanged our ideas with other iGEM teams. Specifically, we joined the iGEM high school tract discussion group. We not only found learnt a lot from other teams, but also contributed a lot of ideas.

To prepare for this meetup, we also made a poster that introduces our project, as shown below. We got the Best Poster Award for this poster.



ExoBlood

A New Method
to Create Artificial Red Blood Cell



Team SHSU_China

Han Yi, Jessica Ji, Luo Yan, Sibao Wang, Shen Jiuqi,
Xinyi Miao, Yuchen Tian, Yunchuan Li, Jiawei Xu,
Tingting Li, Yang Chen.

Our Objective

Create a new type of immune-compatible, easy-to-produce artificial cellular hemoglobin based oxygen carrier (HbOC), that can perform the basic function of red blood cells (RBCs)-oxygen transport.

Project Abstract

We will engineer human cell line to produce exosomes that work as cellular hemoglobin-based oxygen carriers. They can be used in blood transfusion and stroke treatment. We first tried to secrete hemoglobin for oxygen transport inside human cell line HEK293T. Then we focused on loading the protein cargo into the exosome, which we have chosen for the reason of immune-compatibility and easy production. The exosomes will be loaded endogenously with hemoglobin using membrane anchored proteins (CD63) or using exosome-loading pathways inside the cell (WWtag and Ndfip1). By doing this, we will produce an efficient method for future iGEM teams to create protein-loaded exosomes that can be used in therapeutics and develop a potential blood replacement.

What is Artificial Blood?

Artificial blood is a product made to act as a substitute for red blood cells (RBCs). One type of Hemoglobin-based products as artificial blood are cellular HbOCs, in which Hb is encapsulated in a cell-like structure.

Why Artificial Blood?

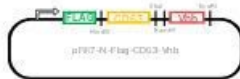
There are complications associated with transfusion of Human RBCs to patients. Such as potential spread of disease, blood type mismatch, lack of supply for rare blood type and storage difficulty. And these are all current problems happening in China. ExoBlood can in theory solve most of the problems listed above, and can potentially be used in stroke treatment due to its size. It can also be modified in the future to have even more functions.

BioGenesis of Cellular Hemoglobin Based Oxygen Carriers by In Vivo Cargo Loading of HEK293T Exosomes

Parts

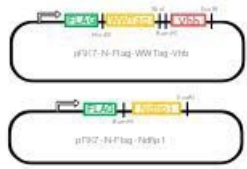
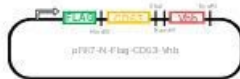
CD63-Vhb Fusion Protein

As previously mentioned, tetraspanin CD63 is a protein used in exosomal membrane protein anchoring. By removing the CD63 stop codon, and attaching the Vhb sequence (BBa_K1321200) after it, we successfully created a vector that can cause human cell line to produce CD63-Vhb Fusion Protein.



WWtag and Ndfip1


By synthesizing WWtag sequence and attaching it to Vhb, the vector can produce a Vhb protein that can use existing exosome loading pathway to be actively loaded into exosomes. Also, we've cloned Ndfip1 sequence from 293TcDNA library so that we can use the sequence to overproduce the key protein in the ubiquitination pathway, which will help the cargo loading process of producing ExoBlood.

Design

We first tried to use human hemoglobin in ExoBlood, but we failed to get the required parts due to complexity and time. So alternatively we used *Vitreoscilla haemoglobin (Vhb)*, which is a kind of hemoglobin used by multiple iGEM teams to increase bacterial survival in low oxygen environments. So we subcloned Vhb from BBa_K1321200 in this year's distribution kit.


To load Vhb inside exosomes, we've used two methods. CD63 is a membrane protein widely exist on exosomal surface, there has been successful attempts on attaching a protein sequence on its C-terminal to load the protein inside the exosomes. WWtag is a 140bp sequence found in viral gag sequence that can label capsid protein to be transported outside the cell. Both method can be used to load Vhb into exosomes.



Vhb Dimer

Experiments & Results

- Subcloning CD63 from manufacturer's vector.
- Subcloning Vhb from BBa_K1321200.
- Cloning Ndfip1 from 293T cDNA library.
- Construct pR17-NFLAG-CD63 (PNC) and pR17-NFLAG-Vhb (PNV).
- Construct pR17-NFLAG-CD63-Vhb (PNCV).
- Culture HEK293T cell for transfection.
- Transfect cells using Liposome PNV, PNC+PNV, PNC V+2,4 plates in total.
- Extract exosome from medium for analysis.
- Use Ripa to lyse normal exosome. Search for key protein (Exosome, CD63) to prove successful extraction.
- Extract exosomal and cellular protein and use Western Blot and FLAG Antibody to analyze result.



What is Exosomes?

Exosomes are cell-derived vesicles that are present in many and perhaps all eukaryotic fluids including blood, urine, and cultured medium of cell cultures. It is now used by synthetic biologist in both therapeutics and diagnostics.

Why Exosomes?

We've used exosomes for the following reasons:

- Because our exosomes are produced by human cell line, it has great immune-compatibility.
- Exosomes can be loaded endogenously, which makes ExoBlood fully functional once extracted.
- Exosomes can be modulated very well using synthetic biology, which makes it a perfect chassis.



IGEM SHANGHAI MEETUP

Written By Yuchen Tian

In order to better prepare for the Giant Jamboree, our team organized a Shanghai iGEM meetup on October 15th. It took place in Fudan University; different teams did their presentations to each other and asked each other questions during the meetup. We also discussed about our projects in detail and gave each other suggestions on how to improve our presentations. We felt more confident about the Giant Jamboree after this meetup.

