POINT OF CARE ANEMIA DEVICE

BME 400 Preliminary Report

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Abstract

Anemia affects twenty-five percent of the global population, being most prevalent in the underdeveloped countries of Africa. Most types of anemia are preventable, but lack of funding and resources for complete blood tests prevents clinicians from making a proper diagnosis and suggesting appropriate treatment. The goal is to develop a cost-efficient, portable, and accurate device to count red blood cells and measure their mean corpuscular volume. A previous team developed a proof-of concept using a microfluidics channel and a computer interface to measure the resistance change of microparticles moving through the microchannel. There are many improvements to be made on the device, and the focus will be on pumping methods, including passive, syringe, and peristaltic pumps, and filtering techniques, including cell filters, a cascading filter, and a built-in microfluidic filter. Each design was evaluated using a design matrix, and the team decided to move forward with the syringe pump and cell filters. Future work consists of further analyzing current microchannels and deciding the appropriate fabrication method of the designed channel. Testing of the design will be done using swine blood and eventually human blood to determine the effectiveness the device in comparison to a Coulter Counter.

Table of Contents

Introduction

Motivation

Anemia is a deficiency of the hemoglobin in the blood, usually characterized by abnormal size, shape, and reduced number of red blood cells and quantified by measuring hemoglobin and mean corpuscular volume. Common symptoms of anemia are fatigue, dizziness, rapid heartbeat, and shortness of breath. [1] There are many types of anemia, but the team's focus is microcytic, normocytic, and macrocytic anemia. Microcytic is characterized by abnormally small red blood cells, usually in the range of 4 to 6 µm in diameter and less than 80 fL volume, and is caused by iron deficiency. Normocytic is characterized by having normal-sized red blood cells (6-8 µm, 80-100 fL) but is caused by decreased production or increased destruction of red blood cells, such as hemolysis. Finally, macrocytic is characterized by abnormally large red blood cells, usually in the range of 8-10 µm in diameter and greater than 100 fL volume, and it is caused by vitamin B12 deficiency or hypothyroidism. [2]

Anemia is very prevalent in underdeveloped countries, due most commonly to malnutrition. Anemia affects roughly twenty-five percent of the global population, but the highest prevalence is in Africa, making up about sixty percent of those globally affected by anemia. Most types of anemia are not only treatable but can be prevented. However, lack of funding and resources for complete blood tests in these developing countries block the ability for clinicians to properly diagnose anemia and suggest treatment. [3] The client, Dr. Philip Bain, volunteers with Global Brigades, an international non-profit organization that helps communities around the world meet their healthcare and economic needs. [4] After volunteering in Ghana, Africa, he wanted to initiate the development of an inexpensive anemia detection device to allow clinicians to make a proper diagnosis and give appropriate treatment options.

Problem Statement

Anemia affects many people worldwide and disproportionately affects those in developing countries due to a lack in medical infrastructure to properly diagnose the blood disorder. A portable, easy to use, and cost-effective device is needed to diagnose the condition in these countries at the time of initial medical care. Anemia can be diagnosed by evaluating red blood cell size using the mean corpuscular volume (MCV). The goal is to fabricate a microfluidic device that effectively measures the MCV of red blood cells to determine if a patient has normocytic, macrocytic, or microcytic anemia with results comparable to current cell counting techniques.

Past Work

Last year, a team developed a proof-of-concept device for this project, consisting of a microfluidics channel and computer interface using LabVIEW. They used a passive pumping method in one inlet-outlet pair to transport 10 µm microparticles through a microchannel, and then they measured the resistance change across the other inlet-outlet pair (See figures 1,2). Each peak in figure 3 represented a microparticle moving from the left side of the channel to the right, while the height of the peak determined the particle's relative size.

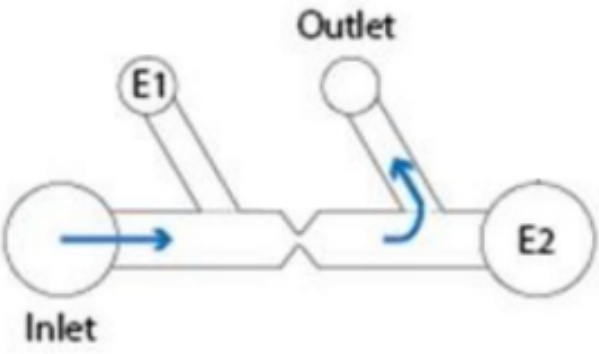

Figure 1: Channel design. The inlet and outlet represent the flow of the microparticles through the channel, and E1 and E2 represent the electrodes. [5]

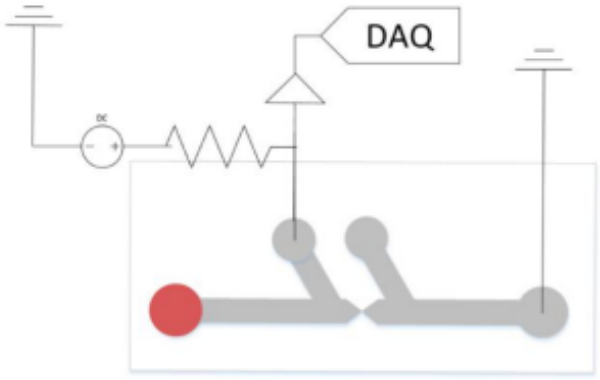

Figure 2: Circuit schematic of the electrodes that measure the resistance change across the microchannel. [5]

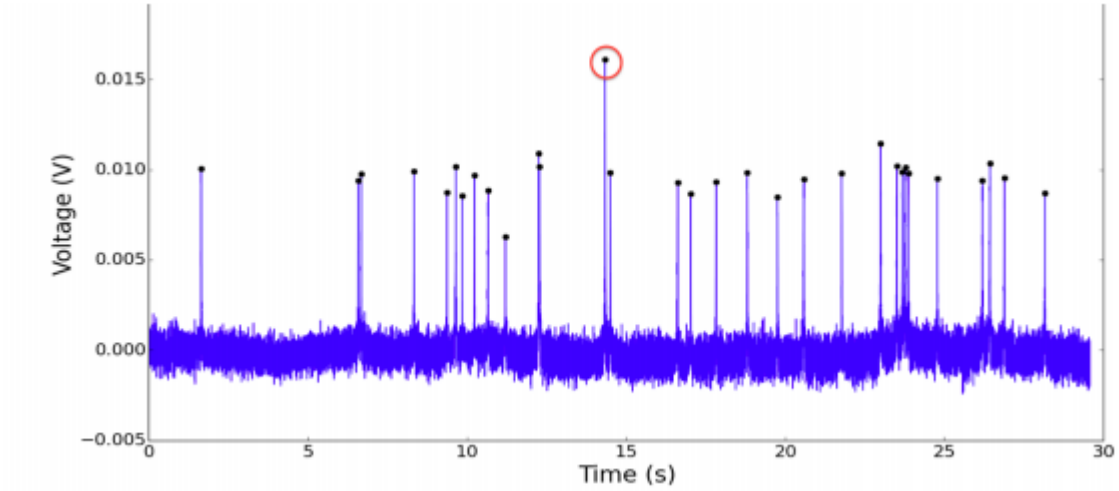

Figure 3: Voltage vs time with coincident event of a microparticle moving through the microchannel. [5]

There is a lot of work left to be done for the device, however. A meeting with the previous team was arranged where potential improvements on their prototype were discussed. They mentioned the difficulty of passive pumping, as adding the proper amount of solution to both the inlet and the outlet for the passive pumping to work is tedious. In addition, they never were able to test whole blood for the device, and a filter is needed for the device to remove unwanted blood cells, such as white blood cells and platelets.

Design Alternatives

In order to continue with the prototype and design of the previous team, two components were evaluated for integration into the design. While the previous team proved the effectiveness of a microfluidic counting channel, blood was never analyzed with the device. Due to blood's heterogeneous composition and high cell concentration, it must go through a preparation process prior to being sent through the channel. This involves filtering the sample, so only red blood cells are being sent through the channel aperture, and diluting to a low concentration so that only one cell passes through the channel at a time. The two design components that were evaluated were pumping and filtering techniques.

Pumping Techniques

The pumping method is necessary to evaluate the best technique to pass the blood sample through the device quickly and effectively.

Passive Pump

The passive pumping technique works by allowing the cells to travel through the channels down their concentration gradient. The counting channel would be filled with PBS or some similar conducting solution. When a droplet of a diluted blood solution is placed on one end of the channel the solution will spread and move to the opposite side of the channel simply because it wants to travel from high concentration to low. An overview of this process can be seen in figure 4.

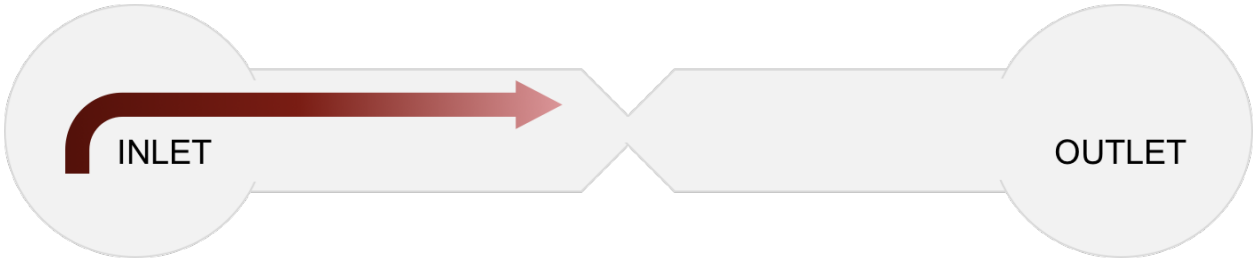

Figure 4: Overview of passive pumping technique. Droplet of blood solution is added to inlet end of channel and blood cells will flow towards the outlet end, down their concentration gradient.

This technique requires very little additional materials. A pipette would be needed to add a droplet of known volume to the channel and that is all. This allows this design to be very low cost per use. There are quite a few disadvantages that accompany this design. It is very difficult to place a droplet on such a small surface and requires a skilled user. This increase in user difficulty can hinder the time it

takes to run the diagnostic test and increases the risk in wasting materials if the clinician is more prone to mistakes. There is also very little control over the rate of flow of the cells through the channel. Additionally, this can increase usage times and decrease the amount of tests that can be performed in some allotted amount of time. Also since there is not any active pumping taking place, the direction of flow might not be unidirectional which would greatly skew results.

Syringe Pump

Using a syringe to manually pass the blood sample through the device is another pumping option. This design includes a small syringe filled with the diluted blood solution and a tubing channel that connects to the microfluidic counting channel. The user would manually compress the syringe to send the sample slowly through the sample. The rate of flow could be measured based on the speed at which the user compressed the syringe and the diameter of the syringe opening where cells will be leaving and entering the channel. The set-up to using a syringe as the mode of pumping blood through the channel can be seen in figure 5.

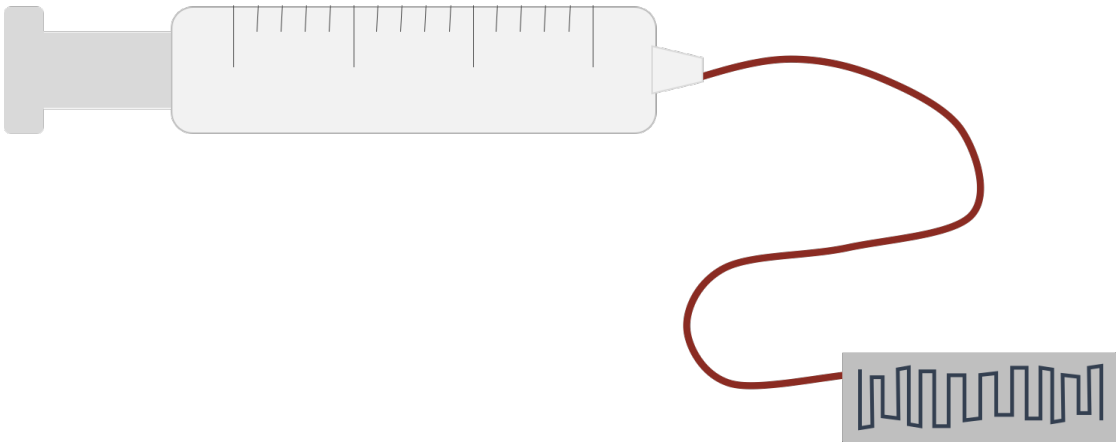

Figure 5: Syringe attached to a microfluidic channel. User would manually compress syringe to send blood sample through the channel. The syringe would be integrated into the channel through an additional tube as shown.

Some advantages of this design is the overall simplicity and ease of fabrication. All components of this pump can be bought commercially and easily integrated into the existing device. Since many clinicians are familiar with operating a syringe, it should also be simple to use. The disadvantages include its tendency for variation between each test. Because the flow rate depends much on the user and the speed at which the user compresses the syringe, this may cause differences in flow between samples. The user would need to apply a continuous pressure at a specified speed to perform the diagnostic test correctly.

Peristaltic Pump

A peristaltic pump to send the blood sample through the channel is a common microfluidic technique and another design alternative that was considered. This pump incorporates flexible tubing and a rotor to send the blood solution through. The rotor compresses the tubing as it rotates pushing

fluid through the tubing unevenly. A peristaltic pump similar to what would be integrated into our design is shown in figure 6.

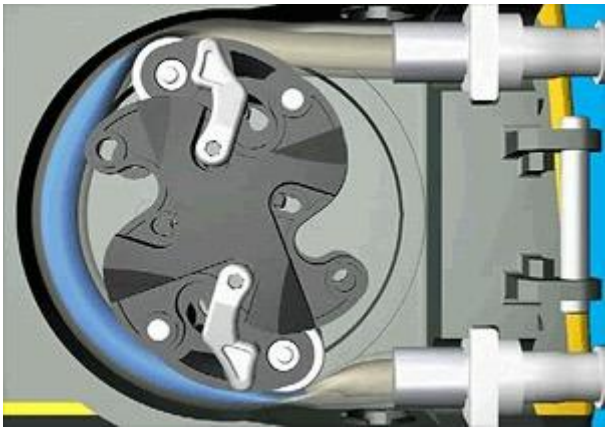

Figure 6: The peristaltic pump tubing and rotor shown here. The rotor rotates, compressing the tubing and sending the fluid (which can be seen in blue) through the channel. [6]

The advantages of using this type of mechanical pump is its automation and ease of use. The pump only needs the user to load the blood sample and the pump will automatically send the fluid through the channel. Because of this automation, the flow rate will also be consistent throughout each test and have no dependency on the user. Because of the pumps mechanical properties and variety of moving parts, this design will require more maintenance than the other options. For use in developing countries where resources are limited, this can be very problematic.

Filtering Techniques

Anemia can be diagnosed by analyzing red blood cell volume and size. Blood is made up of more components than just the desired red blood cells and in order to get the most accurate measurement, a filtering technique is necessary to prepare the sample so that only red blood cells remain. With a filtering mechanism, the goal is to have only RBCs sent through the aperture of the microfluidic counting channel.

Cell Filter

After the cells are delivered into the device via a pumping mechanism, they must be filtered to isolate the red blood cells from the other components of blood. One option is a syringe filter, shown in Figure 7, which contains mixed cellulose ester filter membranes in plastic housings. [7] This filter would be attached to the pump, and cell solution would be pumped through the filter before it is sent through the microfluidic portion of the device for analysis. The filter pore size would be about 11 μm in order to exclude white blood cells, which have a diameter of 12-15 μm, and enable entry of red blood cells, which have a diameter between 4-10 μm. The wide range of red blood cell sizes reflects the type of anemia, where microcytic anemia is displayed by an RBC diameter closer to 4 μm, and macrocytic anemia is manifested by a diameter closer to 10 μm. The diameter of platelets is on the order of 1-3 μm, so they will pass through the filter, but the analysis is not presumed to be affected by their presence.

Figure 7: A syringe filter is connected to the pump and allows for cell solution to be pumped through the filter, removing white blood cells. [7]

This single syringe filter would be easy to manufacture since the filter units are available on the market, and they would only need to be adapted to the pump and microfluidic chip portions of the design using a simple method such as attaching the pump outflow to the filter using a tube and the filter outflow to the chip using another tube. Operation time would be low with this design since the cell solution would only be passing through a single filter. However, this filter would have short lifespan since it is not reusable. In developing countries with few resources, it may be unfeasible to replenish stocks of filters on a regular basis.

Cascading Filter

Another filter option is a multiple-layered cascading filter that would be made up of three filters of decreasing pore size. The filter would be the size of a 5 mL syringe and contain three disks made of polyethylene secured to the walls of the cylindrical syringe, as shown in figure 8. These three disks that contain perforations will allow for successive movement of red blood cells through based on diameter. At the top layer, all but the largest cells (WBCs of ~15 μm diameter) will be allowed to flow through to the layer underneath, and the layer underneath will contain smaller pores that exclude cells of a 13 μm diameter. The final layer would block passage of cells under 11 μm wide, effectively isolating red blood cells from white.

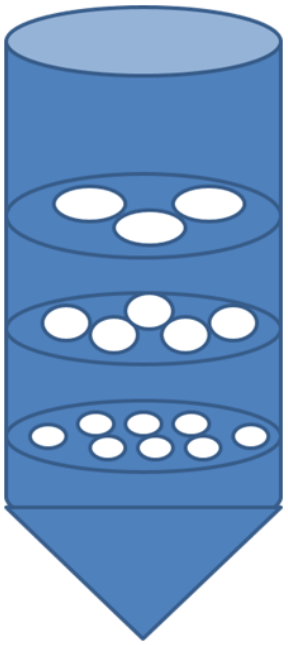

Figure 8: A cascading filter isolates red blood cells by removing larger white blood cells from the cell solution using a succession of three layers, each containing a smaller pore size than the one above.

This design would decrease cell accumulation within the filter by having multiple layers which allow cells to flow gradually through the device instead of building up and clustering together above the filter. However, the design would be difficult to fabricate since this type of filter is not currently available on the market and would have to be built by the design team. In addition, a longer operation time would be necessary with this filter since the cell solution would have to pass through multiple filters instead of just one filter as in the case of the aforementioned cell filter.

Built-in Microfluidic Filter

A final option for the filter is a built-in microfluidic filter that would be incorporated into the microfluidic chip apparatus used for cell analysis built by the previous design team. As shown in figure 9, a row of pillars with 11 μm openings would be constructed on the chip to allow red blood cells to pass through the openings while blocking passage of the white blood cells, which are larger.

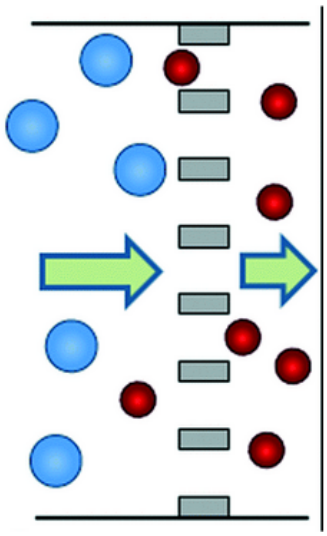

Figure 9: A built-in microfluidic filter consists of channels that allow for passage of red blood cells into the microfluidic device and exclusion of white blood cells. [8]

This built-in filter would be easy to use since it is integrated into the existing device and would allow filtration to be accomplished without using a separate physical component. On the other hand, the filter itself will have to be made on the microscale, suggesting that fabrication will be difficult since much smaller parts are involved. Additionally, white blood cells and debris may clog the filter quickly since there are only a certain number of openings to allow for cells to pass through.

Design Evaluation

Pumping and filtering techniques were analyzed and evaluated based on a variety of categories in order to propose the most effective final design.

Design Matrices

Design Criteria	Passive Pump		Syringe		Peristaltic Pump	
Ease of Use (25)	1/5	5	4/5	20	2/5	10
Time of Use (25)	1/5	5	3/5	15	3/5	15
Cost (20)	5/5	20	3/5	12	2/5	8
Ease of Manufacturing (15)	5/5	15	4/5	12	3/5	9
Size (10)	5/5	10	3/5	6	2/5	4
Safety (5)	5/5	5	5/5	5	5/5	5
Total (100)	60		70 ★		51	

Figure 10: Design matrix for alternative pumping techniques.

A design matrix was created for the three aforementioned pumping techniques (Figure 10). The designs were ranked on the six following categories in order of decreasing weight: ease of use, time of use, cost, ease of manufacturing, size, and safety.

Ease of use is of high priority to our client, Dr. Bain. He said that the experience level of clinicians at point of care clinics in developing countries varies. He wanted our team to design a device that would allow for clinicians of any skill level to use. The passive pumping technique is a difficult procedure to set up, and clinicians who have never used a passive pumping technique before would need to be trained to perform this technique. Although a peristaltic pump is convenient because it can regulate the flow rate, this piece of equipment is expensive and bulky. Therefore, the syringe scored highest for ease of use. Clinicians of varying skill levels have most likely used a syringe before, either for drawing blood or administering shots. They are already familiar with this piece of apparatus, so they would not need any additional training to utilize our device.

Time of use is also of high priority to our client and was weighted the same as ease of use. Dr. Bain said point of care clinics see about 300-500 patients per day, so our device needs to be quick to use. The syringe and peristaltic pump tied in this category, and the passive pump scored the lowest. The previous design team said that it took them a few tries to get the passive pump to work, so that is why it scored the lowest in this category.

The cost category was determined by additional costs to the current device. The passive pumping inlets and outlets are already built into the current device, so no changes need to be made to the current design. The syringe and peristaltic pumping techniques would require additional equipment to be purchased. This caused these two techniques to score lower in this category, with the peristaltic pump scoring lowest because it's significantly more expensive than the other two design alternatives.

Ease of manufacturing was based on how much additional work would be needed to adapt the pumping techniques to the current design. Again, since passive pumping is already part of the current design, it scored highest in this category. The syringe scored second highest because the inlets of the current design would have to be modified to fit the syringe. The peristaltic pump scored the lowest because in addition to modifying the inlets of the current design, the other end of the peristaltic pump would have to be fitted to the blood sample container.

Size was based off of the current device. Therefore, the passive pump scored highest. The syringe scored second highest because it's an additional piece of equipment that needs to be integrated into the current design. The peristaltic pump scored lowest because it is a bulky piece of equipment that is much larger than the microfluidic device.

Safety was not a concern for the three different designs. Clinicians who work in clinics are trained in handling bodily fluids. The three proposed designs do not expose the clinicians to anything they are not already exposed to.

Design Criteria	Cell Filter		Cascading Filter		Built-in Filter	
Ease of Use (25)	4/5	20	4/5	20	5/5	25
Time of Use (20)	3/5	12	2/5	8	4/5	16
Cost (20)	5/5	20	2/5	8	2/5	8
Ease of manufacturing (15)	5/5	15	3/5	9	1/5	3
Size (10)	4/5	8	3/5	6	5/5	10
Lifespan (5)	2/5	2	3/5	3	3/5	3
Safety (5)	5/5	5	5/5	5	5/5	5
Total (100)	82 ★		59		70	

Figure 11. Design matrix for alternative filtering techniques.

A second design matrix was created for the three filtering techniques (Figure 11). The designs were ranked on the seven following categories in order of decreasing weight: ease of use, time of use, cost, ease of manufacturing, size, lifespan, and safety.

Ease of use and time of use follow the same criteria as mentioned in the pumping techniques section. The built-in filter scored highest in these two sections because the filtering component is incorporated into the existing design and no additional equipment is needed. The cell filter scored highest for cost and ease of manufacturing. Cell filters are readily available and can be purchased in bulk

at a low cost. Although microfluidic devices are fairly inexpensive to manufacture, the research and design needed to get to a functional prototype can be quite expensive. Masks needed to fabricate the microfluidics devices can cost over $100 per mask, and it's not guaranteed that our microfluidic filter design would work. The built-in filter will be integrated into the existing microfluidic, so it scored highest in the size category. The cascading filter and built-in filter tied for lifespan because these two designs are reusable. Safety was also not a concern for these techniques as the clinicians should be trained to properly handle bodily fluids.

Proposed Final Design

As seen from the design matrices, the syringe and cell filter scored the highest and will be incorporated into the final design. The familiarity that clinicians have with syringes was a big factor in choosing this design. In addition, syringes are readily available and of low cost. Similarly, cell filters are also readily available and of low cost. Although cell filters are not reusable like the cascading and built-in filter, our team felt that developing countries lack the necessary resources and time to keep the reusable filters cleaned and maintained. Thus, the cheaper and easier alternative is to go with the disposable cell filter.

Future work

The work that needs to be done for this project from this point on can be roughly divided into two main parts: fabrication and development, and testing. Integration of the proposed final design and the previous team's prototype is essential. Finally, testing and evaluating the accuracy of the device as a whole will be necessary to proving the success of the design.

Fabrication and Development

One of the main concerns for this project is taking the microfluidic channel design that the previous group created and perfecting it to make sure that it lines the red blood cells up to pass through one by one and to only flow from the start of the channel to the end without backflow. Before any fabrication will be done, modeling software will be used, such as the software produced by COMSOL. This will give the advantage of being able to test multiple different designs and variables to see which ones will be worth pursuing and fabricating later on, which will help save on production costs down the road.

After modeling, a few key designs will be chosen and depending on budget and time constraints one or several of them will be fabricated and tested further. In terms of fabrication there are many methods currently used to develop microscale devices, two of which are micro-milling and thin layer deposition. Micro-milling is a subcategory of micromachining and is as simple as the name implies, it is milling designs into substrates at very small scales, often times in the microscale. This method is applicable to many different types of materials including polymers, metals and glasses. With the proper tools and experience this method can be used to rapidly prototype designs, however, these tools can be expensive to attain.[9] After discussing with the group that previously worked on this project, it was understood that in their experience micro-milling had a tendency to leave a rough and grooved surface

on the materials that they were using. This had the potential to alter the flow of liquid in the micro-channels, so for that reason it will not be focused on.

The other potential method, thin layer deposition (TLD), also known as thin film deposition, has several useful advantages. This method is essentially characterized by laying down and etching away very thin layers of material, either adding to or subtracting from a substrate. With the proper experience and procedure, it is easy to control the dimensions of the final product and their geometries. This method also has the added benefit of being compatible with a wide variety of materials including polymers, many of which are biocompatible, cheap to attain, and optically transparent. This will help keep production costs down and allow for easy testing of the micro-channels because particles and later on in testing, cells, will be clearly visible under a regular microscope as they pass through the channel. One such polymer is polydimethylsiloxane (PDMS). The group that worked on this project earlier used this particular polymer with general success in developing their micro-channels and a substantial amount remained, which will be used for the continuation of this project.

Another area of focus will be the fabrication and assembly of the blood sample processing portion of the device. This mainly involves the filtering out of white blood cells and the pumping of the remaining red blood cells into the micro-channel. Many of the components for this will be purchased, including cell filters to remove the white blood cells and syringes to pump the sample through the device. The majority of the work for this portion will involve connecting these individual devices together and adapting them or modifying them so that they will interface with the micro-channel. For example, an adapter will need to be devised that will connect the syringe to the channel which may involve some sort of simple hosing system.

Testing

The other main focus of the future work will involve the testing of the device. The first step will be to prove that the device works, making sure that all of its parts work together as they were intended to. This will probably involve a test in which a solution with varying sizes of microbeads are used, each representing a possible particle or cell that may be encountered in a typical blood sample, including red blood cells, white blood cells and platelets. This solution will be run through the device to see whether or not the filter excludes the right sized particles and that the syringe pump manages to push the particles through the whole of the device.

After the proof of concept, an animal model will be used to test the functionality of the device and to determine several key factors about the sample that will need to be used. The most likely candidate will be pig's blood because pigs are often used as models for biological comparisons to human vasculature and it is easily attainable. Once it is determined the device works with blood, it will be necessary to develop a procedure or general outline to perform a test. Various factors and variables will need to be researched and tested, including, but not limited to: dilution factor of the sample, the amount of blood needed to be drawn, the amount of blood cells needed per test, etc. After this is determined, time permitting, human blood samples will ideally be done to compare the accuracy of this device to industry standards such as the Coulter Counter. More research needs to be done, and approval needs to be received to be able to reach this step.

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Product Design Specifications

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Function

Anemia affects many people worldwide and disproportionately affects those in developing countries due to a lack in medical infrastructure to properly diagnose the blood disorder. A portable, easy to use, and cost-effective device is needed to diagnose the condition in these countries at the time of initial medical care. Anemia can be diagnosed by evaluating red blood cell size using the mean corpuscular volume (MCV). The goal is to fabricate a microfluidic device that effectively measures the MCV of red blood cells to determine if a patient has normocytic, macrocytic, or microcytic anemia with results comparable to current cell counting techniques.

Client requirements

- Device should provide an accurate diagnosis of anemia and differentiate between microcytic, normocytic, and macrocytic anemia
- Device should be low-cost and adaptable to resource-limited environments
- A clinician should be able to use the device easily and reliably after proper training with an intuitive user interface
- The device should be able to diagnose anemia at the point of care

Design Requirements

1. Physical and Operational Characteristics

a. Performance requirements: The device should be able to diagnose anemia, by identifying patients' Red Blood Cell (RBC) count, Mean Corpuscular Volume (MCV), and hemoglobin (Hb) levels. Testing and diagnosis time should take less than 30 minutes.

b. Safety: Blood samples must be introduced to the device in a contained environment so that no contamination occurs between device uses and between the user. The device must be used with proper blood collection techniques.

c. Accuracy and Reliability: RBC, MCV, and/or Hb levels should be measured with at least 95% accuracy when compared to standard counting techniques (i.e. Coulter Counter) to allow for diagnosis of anemia. Diagnosis with 95% accuracy should take no longer than 30 minutes.

d. Life in Service: Device hardware should function for at least 5 years, and the blood collection platform will be reusable. Software should be able to be upgraded when necessary.

e. Shelf Life: Device and all attachments should have the ability to be stored for 5 years from the time of manufacture.

f. Operating Environment: The device will be primarily used in developing countries (i.e. countries in Africa, Southeast Asia and South America), so available resources should be taken into consideration. Generally access to most resources is limited so the device should be able to stand alone or run with minimal outside help (i.e. batteries or a small generator).

g. Ergonomics:The device should be easily transportable and usable by clinicians of varying experience and educational backgrounds.

h. Size: The device should be small enough to fit on a benchtop in a clinical setting while maintaining enough portability to transport to areas in need. The circuitry housing should be no larger than 4" x 6" x 10". The microfluidic device should be no larger than 4" x 4".

i. Weight:The device should weigh no more than five pounds (2.3 kg).

j. Materials: Materials should be low-cost and durable. They should also be biocompatible as to not disrupt cell structure as the blood is moving through the device (i.e. PDMS for the microchannels).

k. Aesthetics, Appearance, and Finish: Device should have an output screen to view RBC, MCV, Hb levels, and classification of anemia. Data should be easily interpreted by the user. Circuitry should be hidden from view.

2. Production Characteristics
a. Quantity: One prototype is needed for proof of concept.

b. Target Product Cost: Product should not cost more than $200.

3. Miscellaneous
a. Standards and Specifications: To be determined at a later time. Device will be used in developing countries, not covered by the FDA. FDA guidelines will be followed, but approval is not needed.

b. Customer: Clinicians of varying skill levels in developing countries.

c. Patient-related concerns: Should be able to give patient a diagnosis at point of care.

d. Competition: Existing devices include the following:

- Coulter Counter: A coulter counter measures the MCV, hemoglobin and the red blood cell distribution width. The coulter principle, which uses the direct current impedance method, governs the use of the device.

- HemoGlobe: HemoGlobe is a non-invasive device designed by students from Johns Hopkins University that measures the hemoglobin level of a blood sample. The device measures the parameter and then sends the data to a center for further data analysis.
- Microfluidic card for RBC analysis: Patent - US8034296 B2: The cartridge enables a complete blood count including red blood cell analysis of various parameters. The cartridge channel may also be subject to an electric or magnetic field during operation.