A microcontroller based microfluidic biochip for calcium percentage detection in blood

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Article Info

ABSTRACT

Apheresis is an essential step in clinical diagnostic. Searching for a safe and fast way was necessary to avoid wasting time and obtain the desired results efficiently. This research includes two stages: First, a microfluidic biochip with the properties of fast processing response, automation capability, and low-cost was designed having a tiny mesh-type channel that filters pure plasma from the blood components and a straight channel to deliver the pure plasma to a tank. Second, an automated system was designed to detect blood calcium levels using a microcontroller and a colors-detection sensor TCS3200-DB. The device is designed to take a 0.15 to 2.15 mg/dL, 10-160 µm blood sample, which is considered small compared to the samples taken for the blood apheresis process used in laboratories where a substantial quantity of pure plasma is obtained naturally. Pure plasma is mixed with calcium detectors R1 and R2 to get a violet-colored solution with a wavelength between 390 nm to 440 nm. The results of the proposed device were compared with the traditional methods used spectroscopy method using concentrations of 10 different blood samples, and the results proved that there is a slight error between the two processes.

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1. INTRODUCTION

At present, there are many challenges that the elderly must face, especially after their remarkable increase in numbers globally. Among these challenges is osteoporosis, especially among females, as they suffer from symptoms of this disease after menopause piecewise modification operation (PMO). Osteoporosis is considered one of the most dangerous diseases in the current era. It is asymptomatic or called "the silent disease" It generally appears in the hip, spine, femur, or wrist areas in the form of a fragile tear. Then the condition begins to develop slowly without the patient noticing it [1]. The World Health Organization (WHO) defines osteoporosis as "a systemic skeletal disease characterized by low bone mass (measured as bone mineral density (BMD)) and micro-architectural deterioration of the bone tissue with a consequent increase in bone fragility and susceptibility to fractures, involving the wrist, spine, hip, pelvis, ribs, or humerus" [2].

The WHO has defined osteoporosis as a non-communicable disease. Still, at the same time, it is considered one of the leading and dangerous diseases as it causes a large number of deaths among the injured, as 20% of females with hip fractures die within one year of the fracture. Also, 50% of them cannot

fully restore their functional activity. Studies have also shown that people with vertebral fractures may suffer death in large proportions or suffer from functional impairments [3].

Many methods are available to detect osteoporosis according to the examination method used, where the detection methods are either based on different imaging devices such as X-rays and CT scans [4]-[7] or based on the detection of calcium in the blood in vitro [8]-[11]. The whole human blood consists of two main parts: the first part is red blood cells and platelets, which make up 45% of the volume of the first part, and white blood cells, which make up 1% of the volume of the first part of human blood. The second part of human blood consists of blood plasma, which constitutes approximately 54% of human blood volume as a whole. Blood plasma consists of 91% water, 7% proteins, 2% inorganic ions, and a balanced percentage of a group of organic substances such as globulin, nucleic acids, and albumin [12]. Unfortunately, the blood cells and cellular components that remain in the blood interferes with any biomarkers detection processes when using traditional diagnosing system test, so it is essential before any testing procedure to separate the blood contains from plasma so these tests can be done with a cell-free serum [13], where usually blood separation techniques are perfumed in a laboratory using centrifugation equipment [14].

Microfluidics science was developed for biomedical, biological, and chemical applications with a reasonable enabling technology. The present techniques used for separation methods in fixed microfluidic systems are classified into two types: active or passive systems. In the active system, the separation of species depends on outside applied force fields such as the acoustic separation [15]-[17], magnetic force separation [18], [19], and the dielectrophoretic separation [20]-[22], but in the second case, there was no need for an external force to be applied. The passive techniques were likable between these two methods primarily because they have a more straightforward and more accessible design than the active method, a more uncomplicated integration with biosensors, and a lower cost [23]-[25]. Microfiltration is one of the techniques used in passive systems, which depends upon the size exception effect to delay the movement of the cell, which can be considered one of the more effective methods [26]-[28].

In this research, we designed a passive type microfluidic biochip to separate pure plasma from the rest of the contents of the blood sample based on the characteristic of the effect of volume exclusion using the mesh type plasma filtration channel and the delivery of the pure plasma to a special tank away from the rest of the sample components, which is a method that differs radically from sedimentation methods using the standard centrifugation [29], where traditional methods store the resulting plasma in the same tank with the rest of the blood contents. After completing the plasma separation process, the required test can be easily performed without secondary treatment. Because of the high advantages of this method, it can be considered to improve the proposed system to work according to point-of-care (POC) applications.

2. RESEARCH METHOD

This research was divided into three main axes: In the first axis, we designed the medical chip as mentioned previously in the Introduction section with the addition of appropriate reagents used in calcium detection tests, the second axis ensures the process of detecting calcium in the blood sample based on a microcontroller and the color sensors attached to the designed system, where the percentage of calcium in the sample under test is identified based on the wavelength of the solution produced after the plasma mixing process with the reagents and compared with the database stored in the memory of the microcontroller. The results are displayed on the screen of the proposed device (standard, high, or low). The third and final axis In order to ensure the validity and accuracy of the proposed system, the results obtained from the system were compared with other results of the same samples that were measured using the standard spectroscopic method.

2.1. Design of the microfluidic biochip

The proposed biochip consists of two main parts: an upper part made of PDMS material that includes a blood sample inlet and a connected nanochannel. The lower part, made of glass, contains mesh channels for plasma filtration and a collection tank. Figure 1 demonstrates the schematic of the proposed biochip. The blood sample is released to the microfluidic biochip inlet towards the 2 µm depth mesh channel used for plasma filtration by the effects of the capillary forces. The blood cells can be eliminated through the microchannel by the blood sample flow in the chip. The plasma is being separated from the whole sample via the effect of size-exception into the filtration channel, and the pure plasma flows into the 500 µm depth tank drilled in the glass layer of the designed biochip.
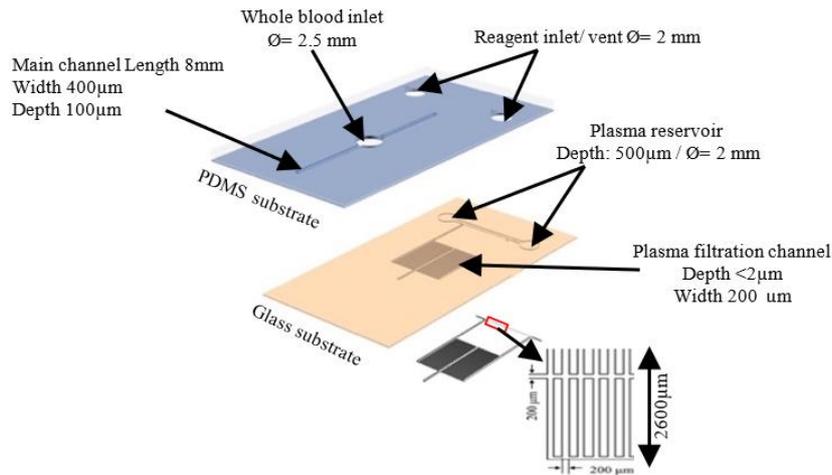
Figure 1. Schematic of the proposed polydimethylsiloxane (PDMS) biochip for blood plasma separation

2.2. Fabrication of the microfluidic bio-chip

The traditional photolithography and wet etching techniques were used to manufacture the microfluidic biochip. These processes are the same as those described in the study by Kuo and Zhan 2015 group [30]. However, the pattern of the photomask of the biochip was created by using an auto-CAD software package. The thin layer used was made from a SU-8 photoresist spin-coated on a silicon material and patterned using the lithography techniques Figure 2. The final polydimethylsiloxane (PDMS) substrate design was made by transferring the resulting SU-8 microchannel designed as a model. A 2.5 mm diameter hole was dug in the layer of PDMS using a less sharpened needle to create the blood inlet of the microfluidic biochip. The glass material was used to spin-coated the photoresist S1818 and was softly baked at 96 C° for a time of 3.5 min. This photoresist was exposed to U.V. light using the plastic mask, where MP351 solution was used to develop and was baked at 125 C° for 13 min. The glass substrate was soaked for 2.5 min into buffered oxide etchant substance BOE 6:1, J.T. Baker [31]. The necessary form of grating-type plasma filtration channel was made in the depth of 1–2 µm.

After completing the process of etching for the tank and microchannels in the chip and before the sticking processes between the PDMS layer and the glass layer, we cleaned all the remaining impurities from the glass surface of the chip using acetone solution. Then we placed the chip in a hydrochloric acid solution with a volume of 1 molar for 25 minutes. Finally, we applied the glass layer of the slide to the PDMS layer and glued them tightly with oxygen plasma adhesive [32], [33].
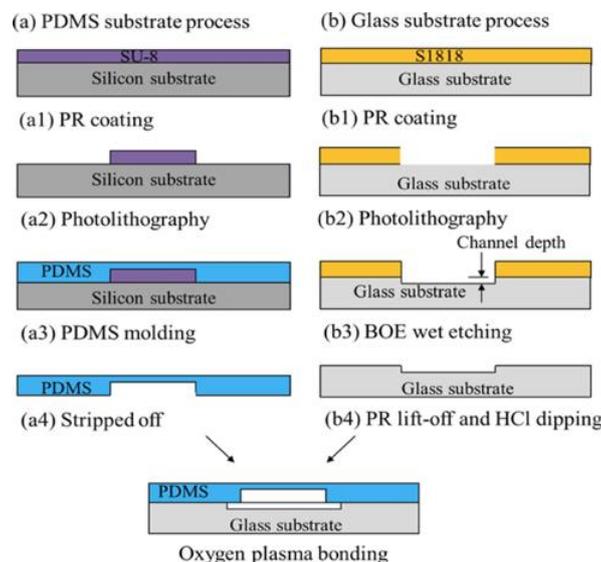

Figure 2. The microfluidic bio-chip fabrication process

2.3. Microcontroller arduino UNO

A microcontroller is a free hardware platform consisting of an integrated circuit that can record instructions. These instructions are written with a particular programming language that allows users to set programs that interact with electronic circuits [34], [35]. The arduino UNO microcontroller used in this paper as shown in Figure 3 contains an ATmega328 board with 14 input and output pins. These pins can be used as six pulse-width modulation (PWM) outputs, six analog inputs, a USB connection, a power jack, a 16MHz crystal oscillator, a reset button, and an ICSP header. This microcontroller is connected to the computer via a USB cable. Also, it can be supplied with an AC or DC adapter to start [36]. These specifications make this microcontroller suitable for this project. Figure 4 shows the flow chart of the microcontroller driver software. The microcontroller will process the signals received from the detectors. After that, the set values will be sent to the LCD screen attached to the system for display.
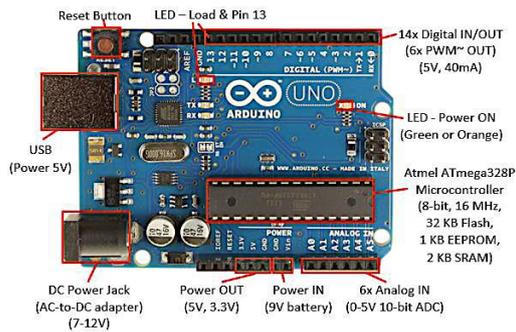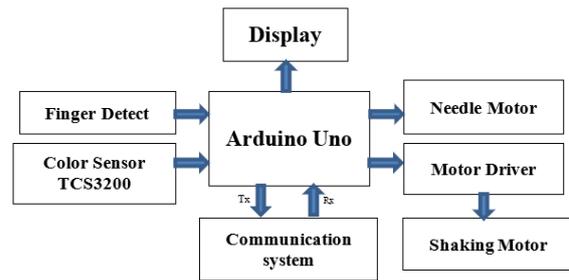
Figure 3. Arduino UNO microcontroller

Figure 4. The operating program of the microconteroller flow chart

2.4. Color sensor TCS3200-DB detector

The color sensor detector (TCS3200-DB) is a full-color detector that contains a sensor chip (TAOS TCS3200 RGB), a collimating lens, stops to set the optimal sensing distance, and white LEDs. This sensor can be connected in two ways, either directly to the motherboard (BASIC Stamp-2pe) or interface to any module (BASIC Stamp) as an optional module for DB-Expander SIP adapters [37]. The TCS3200 color detection sensor as shown in Figure 5 can measure an unlimited range of visible colors based on photodetectors equipped with a set of standard filters (red, green, blue) or without a filter. These filters or filters are evenly distributed for all colors throughout the array in order to eliminate site bias. The sensor also contains an oscillator that produces a square wave as an output with a frequency proportional to the distance between the desired color [37].
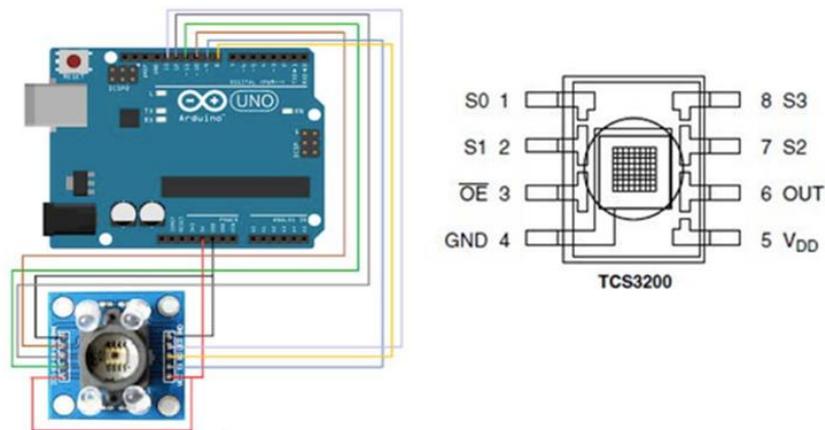
Figure 5. TCS3200 chip schematic with pin map

3.   RESULTS AND ANALYSIS

Calcium is the 5th most mutual element and the general cation found in the human body. Calcium can be found in 3 physiochemical statuses in the plasma; bound, complexed with some small anions, and free-ionized. In order to avoid the effects of protein interference in the blood sample with the reagents used, Calcium+ tests mainly use pure plasma samples. Usually, the calcium test takes about 3-5 minutes in normal conditions using the spectrophotometric method [38], but the process will take no more than 2 minutes when we use the biochip. At first, the needle connected to the proposed device Figure 6 will take the order from the microcontroller to prick the patient's finger and automatically deliver the blood sample to the chip. Then the chip will separate the plasma from the rest of the blood contents from the blood sample, as mentioned previously. The obtained pure plasma will automatically flow to tank A, which contains calcium reagents R^1 and R^2 stored in calculated proportions [39]. The mixing processes of the plasma with the reagents will take time between (4-6) sec to ensure good mixing by using the shaking motor.

A resultant violet solution mixture will be exposed to the color sensor. The sensor will send a signal to the microcontroller with the exact wavelength of the mix. The microcontroller will compare the wavelength amount with the stored database, previously taken from the manual tests. It will provide the results as normal, hyper, or hypo as in Table 1. The last stage of this research included comparing the results obtained from the proposed system with the results measured using the traditional method (refer to Table 1) to calculate the percentage of calcium in the blood. Samples were taken from ten donors (six females and four males) of different ages (21-60 years). There was a slight difference between the two reading methods due to human errors. Figure 6 shows the schematic diagram of the proposed system in this paper. Finally, the results will be sent to the responsible doctor using the communication GSM system attached to the device for remote patient monitoring.
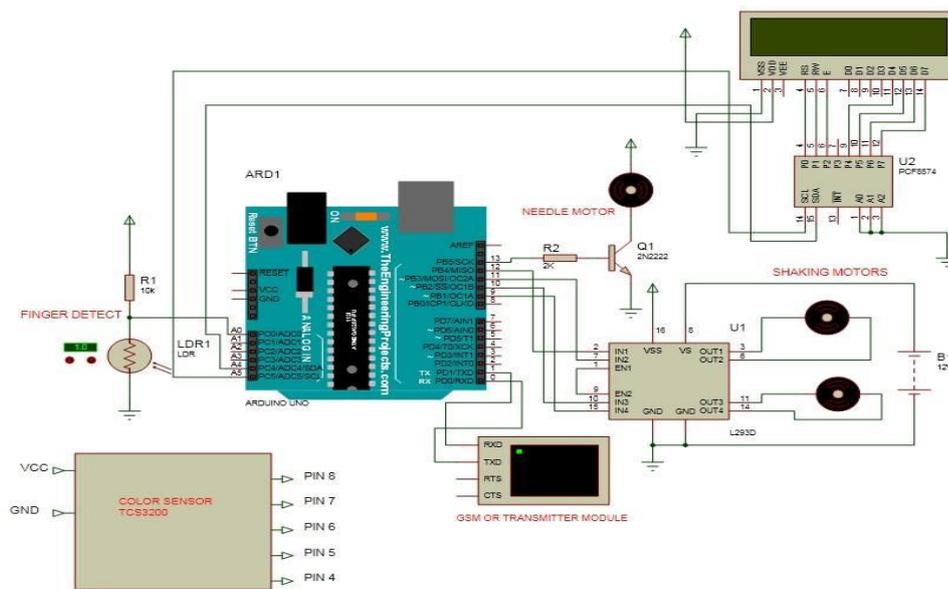

Figure 6. Schematic diagram of the designed system

Table 1. Difference between the results taken from the spectroscopic method and the designed method

	Sex	Age	Ordinary test with the spectroscopic method	Status	Tests with the proposed biochip
1	M	21	10.9 mg/dl	Hyper	Hyper
2	F	30	10.2 mg/dl	Normal	Normal
3	F	39	10.05 mg/dl	Normal	Hypo
4	M	50	9.8 mg/dl	Hypo	Hypo
5	F	43	10.1 mg/dl	Normal	Normal
6	F	33	10.3 mg/dl	Normal	Normal
7	F	60	9.5 mg/dl	Hypo	Normal
8	M	45	8.6 mg/dl	Hypo	Hypo
9	M	53	8.8 mg/dl	Hypo	Hypo
10	F	37	9 mg/dl	Hypo	hypo

The device automatically delivers 5.0 µL of a blood sample to the biochip inlet for each test. The plasma in the collection tank normally flowed until the pressure uilibrium inside the tank was obtained, then the plasma flow stopped. An equal amount of reagents R1 and R2 is stored in the tank to be mixed with the plasma produced under diffusion phenomena. After the blood sample was entered into the chip, the plasma began to collect inside the tank after three seconds of starting the process, and the time required for the start of the plasma reaction with the reagents was 14.5 seconds. On the other hand, the mean clotting time was calculated as 13.7 seconds with a standard deviation of 0.85.

As mentioned before in the designed biochip, the depth of the filtration channel is 1.67 µm. Hence, the track was able to prevent or filter out all blood substances red blood cells size of 8 µm, white blood cells size of 10 µm, and platelets size of 2.5 µm, allowing only the plasma to get through. The plasma tank was almost full within 2 min. The proposed biochip extracted near to 1.94 µL of plasma in the tank. The flow rate average over the full filling time was near 0.01 µL/s. We took a small amount of plasma produced in the biochip and examined it using an optical microscope to verify the chip's efficiency. The results showed that the concentration of cells remaining in the plasma after the separation process was less than 0.15%. The proposed system can be considered a practical and straightforward solution to test the proportion of calcium percentage in the blood sample. The database used and stored in the proposed system was taken from 250 tests using the spectrophotometric method, where we read the wavelength of each test, and it was stored in the form (normal, hyper, and hypo) as in Table 2.

Table 2. Database for the proposed system the results were taken from 250 test using the spectroscopic method

	Wave length	Result
1	390-405 nm	Normal
2	406-430 nm	Hypo
3	431-440 nm	Hyper

4. CONCLUSION

An automated system for calculating the proportion of calcium in the blood has been proposed that contains a PDMS biochip based on microfluidics with superficial characteristics used to separate the plasma from the blood sample and test the proportion of calcium in the blood. The proposed biochip includes an upper PDMS layer comprising a sample inlet port, a vertical micro-channel, a mesh-type plasma filtration channel placed in a substrate glass layer, and a plasma collection tank. The second part of the proposed regulator includes connecting a microcontroller to the chip, depending on the color sensor. It will detect the calcium percentage in the blood sample after comparing it with the database stored in the device.

The test results showed that the extracted plasma started to reach the collection tank 3 seconds after the test with a flow rate of 0.01 µL/sec, while the concentration of remaining cells in the extracted plasma was less than 0.1%. Also, the time required for the blood sample to clot was equal to 13.4 seconds, and the total time from the beginning of the test until the result appeared on the screen was only 2.5 minutes. This research paper presents the idea of a new model for an accurate, fast, and effective diagnostic system for measuring the proportion of calcium in the blood, where this process can be performed without the need to return to specialized clinics or consult an expert in clinical analyzes and at any time possible.

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