Lipid Accumulation in Vessel-imitating Tubes under Microgravity Condition

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Table of Contents

Experiment background

1. introduction

The Coronary Arteries play a very important role in heart health because they deliver oxygen-rich blood to the heart muscle. Hence, any kind of Coronary Artery disease or accumulation of plaque will reduce nutrient and oxygen flow to the heart and result in a heart attack or even sudden death.

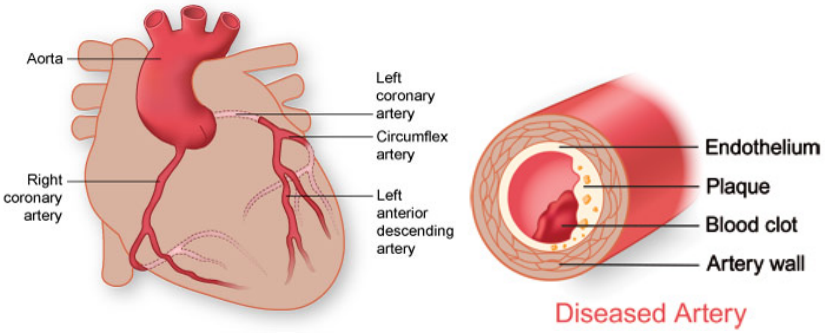


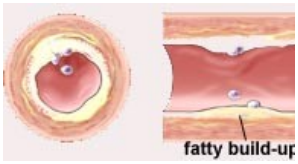
Fig.1 Coronary Vessel

In this research, we aim to study a progression of coronary atherosclerosis or Coronary Artery Disease (CAD) in the phase of lipid accumulation which plays a crucial role in the progression of the disease under microgravity.

Firstly, the process of coronary artery disease (CAD) has been studied which concluded in the following content. This research will focus on the first stage of the process which the gravity may play role in it.

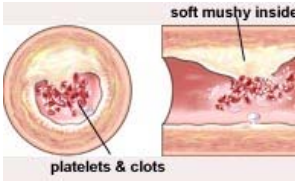
1.1 The process of coronary artery disease

1. Coronary artery disease starts when the blood vessel walls begin to show streaks of fat.



2. When the fat builds up, it is causing slight injury to the blood vessel walls. Then the fat and other substances combine to form a material called plaque.

3. Over time, the inside of the arteries develop plaques. Many of the plaque deposits are soft on the inside with a hard fibrous “cap” covering the outside. If the hard surface cracks or tears, the soft, fatty inside is exposed. Platelets come to the area, and blood clots form around the plaque. This causes the artery to narrow even more.



4. Sometimes, the blood clot breaks apart, and blood supply is restored. In other cases, the blood clot may totally block the blood supply to the heart muscle, called a coronary thrombus or coronary occlusion - causing an acute coronary syndrome.

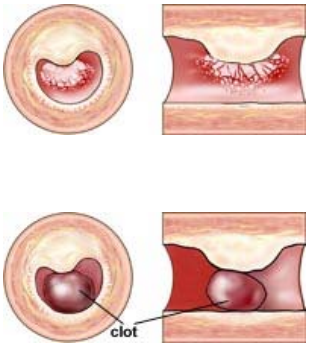


Fig.2 The process of CAD

1.2 Accumulation of low density lipoprotein

Accumulation of low density lipoprotein in the inner layer of the arterial wall is believed to lead to the Coronary Arteries disease (CAD) [1]. The inner layer of the arterial wall, dense extracellular matrix (ECM) forms a tight network [2]. Some ECM such as decorin has negatively charged which can be interacting with LDL which has positively charged [3].

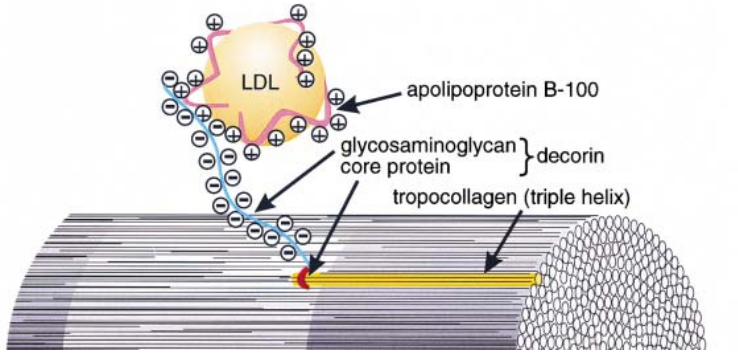


Fig.3 This figure presentation of the interactions among low-density lipoprotein (LDL), decorin, and a collagen fibril in real vessel.

1.3 Particle motion

LDL which causes the CAD is a particle in the blood. Gravity affects to its motion, for example, fluid flows in horizontal tube, consider a particle of mass *m* moving through a fluid under the action of an external force F_e

$$m\frac{du}{dt} = F_e - F_B - F_D$$

u = velocity of the particle relative to the fluid

F_B = buoyant force on the particle ($F_b = mr\, a_e/r_{\,p}$)

F_D = Drag force ($F_D = C_D u^{2r} A_p/2$)

Then

$$\frac{du}{dt} = a_e - \frac{a_e \rho}{\rho_p} - \frac{C_D u^2 A_p \rho}{2m} = a_e \frac{\rho_{p-}\rho}{\rho_p} - \frac{C_D u^2 A_p \rho}{2m}$$

Motion in gravity force (a = g)

$$\frac{du}{dt} = g\frac{\rho_{p-}\rho}{\rho_p} - \frac{C_D u^2 A_p \rho}{2m}$$

Under normal gravity

$$\frac{du}{dt} = \frac{C_D u^2 A_p \rho}{2m}$$

Under micro gravity

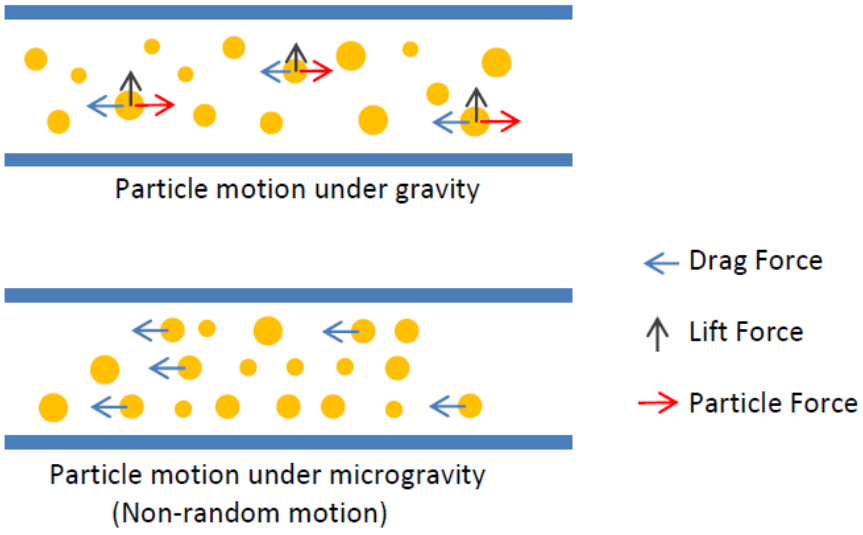


Fig.4 The effect of particle motion of horizontal tube for fluid transport phenomena of horizontal tube under microgravity

The equation above shows that, in microgravity (g ~ 0 m/s^2) particles are non-random motions, so that particles may keep moving in certain directions.

Gravity affecting to amount of cholesterol accumulation on a vessels' inner wall is depends on direction of vessels and fluid's flow.

1.4 The importance of being under microgravity to this experiment

All in all, after we understood all the basis theories involving in the progression of CAD. We realized that one important parameter is an accumulation of lipid on the surface. We concluded our hypothesis that effected particle motions affect a coagulation of lipid and lead to the change in lipid accumulation on the surface.

This hypothesis infers many useful things in developing of medical science, bio-engineering and living in space healthcare, a heart disease tendency of astronauts, a possibility and a benefit growing a transplant heart in space, for instances. And this study would help us answer all of these questions.

2. Purpose

To accomplish the experiment to studying how gravity affects the surface accumulation of cholesterol flowing in a vessel like coronary arteries. These accumulations naturally lead to atherosclerotic plaques forming in coronary arteries' surfaces and it is one of the major causes of hypertension and heart disease. The zero gravity will be generated by parabolic flight where our experiment will be conducted.

3. Expected results

3.1 Quantitative data of the difference of cholesterol accumulation on the surface, one under normal gravity and another one under microgravity.

3.2 The explanation of “How does gravitational changing affects to the accumulation of cholesterol on the surface?”

4. Applications

In case that micro gravity reduces cholesterol accumulation: The study will show us the potential of coronary arteries tissue culturing under micro gravity which would perform a better result (less threatening cholesterol accumulation) than on earth.

Vice versa, in case that micro gravity induces cholesterol accumulation: It will lead us to consider a health effect of microgravity for astronauts, which is CAD in this case. Hence the solution to cure cholesterol accumulation under this circumstance must be looked for.

Materials and methods

The experiment setup consists of three main parts as **Fluid pumping system** (representing as "a heart"), **Artificial blood vessel (AB-Vessel)** mocking up (representing as "the coronary artery"), and **Artificial Blood solution (AB-Solution)** mocking up (representing as "blood").

The parameters which vary in this experiment are the most lipid accumulation involving parameters respect in term of physical. Though as **need of simplicity of the experiment**, the experiment setup cannot imitate all the parameters in human body due to its complexities.

1. Experiment/test item

1.1 Fluid Pumping System

1.1.1 Microcontroller

1.1.2 Stepped motor

1.1.3 Driver stepping motor board

1.1.4 Relay 6-12 V

1.1.5 Power supply 110 to 5V,12V (Switching, Adaptor)

1.1.6 Air pump

1.1.7 Pneumatic Valve 3/2 or 5/2 (Solenoid 6-12 V)

1.1.8 Speed Control Valve

1.1.9 Syringe 25 cc.

1.1.10 Valves

1.1.11 Linier state

1.1.12 Blood bags

1.2 Artificial Blood vessel (AB-Vessel)

1.2.1 AB-Vessel’s inner layer: Silk scaffold tube

1.2.2 AB-Vessel’s outer layer: dipped polymer

1.3 Artificial Blood solution (AB-Solution)

1.3.1 Total cholesterol (Sigma)

1.3.2 Triton X

1.3.3 Buffer (Sigma)

2. Experiment design

2.1 Fluid Pumping System

It relies on the driving system with a motor, syringes and AB-Vessel. By driving a motor through the driving system, we can control flow rate of AB-Solution feeding from the syringe. Syringe will be set in vertical and contain AB-Solution 20cm^3 and Air 1cm^3. Syringe will feed AB-Solution only in microgravity stage and feed air to eliminate solution from AB-Vessel because solution which still in AB-Vessel in 2g stage may cause result's error.

Generally in the body, blood flow is laminar [4] but a flow entering an AB-vessel is not laminar flow until the fully developed flow begins. The length of the tube between the start point and the point where the fully developed flow begins is called **the entrance length**.

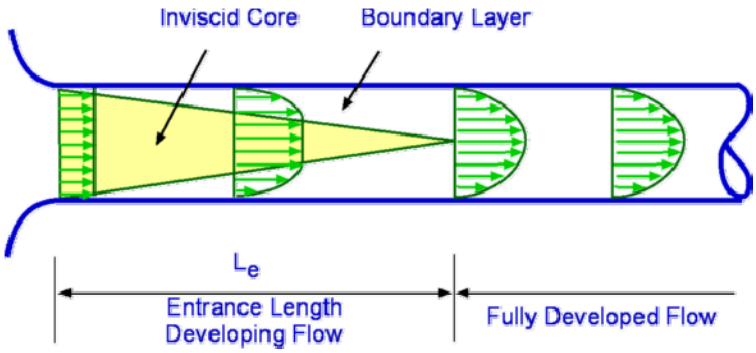


Fig.5 Flow at the entrance to a tube

In our experiment, Reynolds number is 176, calculate from equation for Reynolds number (Re) [5] with flow rate 80 ml/min [6], vessel diameter 3 mm [7], blood viscosity 3.2 cP [8], and room temperature 25 C^o. Reynolds number is used to calculate the entrance length; it is and equal to 3 cm.

Fig.6 diagram of the mechanical design.

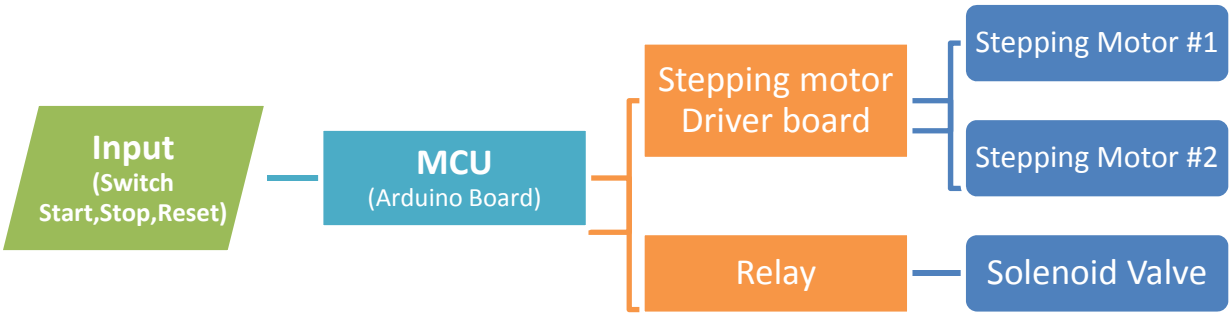


Fig.7 Functional diagram

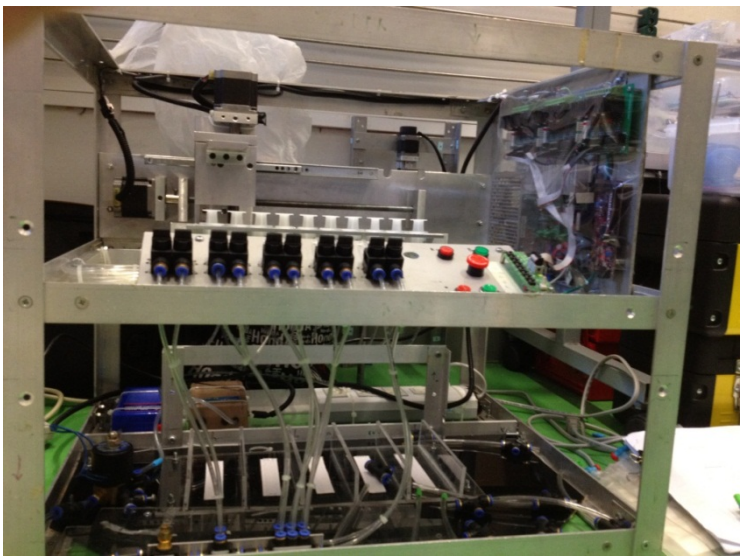


Fig.8 Machanical part of experiment

2.2 AB-vessel's inner layer fabrication : Scaffold

In our experiment, inner layer of AB-vessel will be fabricated from silk scaffold to represent the vessel extracellular matrix (ECM). Silk scaffold has negatively charged [9] like the real vessel ECM, easy to fabricate and has more surface area compare with the smooth tube. The inner diameter of AB-vessels is 3 mm close to human's left ascending coronary artery average dimension. [7]

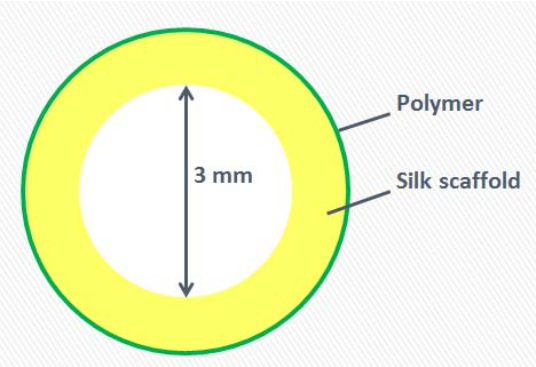


Fig.9 Diagram of AB-vessel

Fabrication of silk scaffold tube starts with the solvent solution was prepared by ratio of $CaCl_2$: Eth : H_2O = 10 : 7.8 : 9.6. After that, silk fibroin was dissolved with the solvent solution. Then the solution was evaporated until became sticky. Finally, tube molds were used to fabricate tube shape scaffolds.

In this experiment, outer side of silk scaffold tubes has to be coated to avoid leakage problem. Coating material was Polyurethane dispersion in solvent (Astacin Finish LP (BASF®)).

First, a stainless steel rod (3 mm. in diameter) was inserted into a scaffold tube. Second, a heater was used to heat scaffold to reduce moisture that affect the coating process. After that, the specimen was dipped in a polyurethane bath and heated until polyurethane coated was dry. Doing the same method, dip and dry, until half way of dipped process, attach the connection tubes at the end scaffold tube. Then continued dip and dry until the specimen reach its final thickness. Finally, remove the stainless steel rod from the coated scaffold tube.

2.3 AB-solution : Cholesterol solution

AB-solution was used in this experiment instead of real blood.

To prepare AB-solution, the 300 mg of Cholesterol with water surfactant (tritan x) and buffer in the ratio of 1000 ml : 100ml : 50 g was mixed together and stirred for 5 minute.

On board experiment procedure

Before the flight, experiment procedure, manual operation in case of emergency situation and experiment condition were designed.

For compare the difference of cholesterol accumulation on AB-vessels inner surface between normal gravity and microgravity condition, on ground and on board experiments used the same experiment procedure.

Table 1 On board experiment procedure

State of aircraft	G state	Work item	Time required
After aircraft taking-out from hangar and before engine start	Ground	without power supply	About 20 minutes
Engine start (No power supply during this time) ↓	1G	-	About 5 minutes
Takeoff ↓ Experiment airspace arrival	1G	-	20 minutes to K airspace 35 minutes to G airspace
" 2 minutes before starting 1st PF" ↓	1G	Switch on main power-the power LED should be power on	1 minute
"1 minute" ↓	0.5 - 1.2G	Turn on air valve set 1 by anti-clockwise for 90 degree At microgravity	30 seconds
"30 seconds" ↓	2G	-	10 seconds in 0.5-1.2G 20 seconds in 2G

"NOW"	µG	push the start button on the remote(operate at seat)RUN LED should be on and y-axis motor will start to turn till syringe were empty	20 seconds
End of µG ↓ Normal flight	1.5G	-	20 seconds
The following experiment preparation ↓ (Returns to "2 minutes before 2nd PF" call. And repeat PF till predefined times.)	1G	- Closed the sample bag and air valve - Open next set air valve - back to seat wait for next microgravity	Fill in required time to prepare experiment for next PF minutes
Departure from experiment airspace ↓ Landing	1G	-	20 minutes from K airspace 35 minutes from G airspace

Manual Operation in case of emergency situation

1) Switch on air pump II (operate on battery)
2) Open air manual valve (red lever)
3) While microgravity push the syringe 15 second
4) Open air valve for 5 second
5) Close sample bag valve
6) Repeat from step 3-5 until last set
7) Turn off air pump II and manual air valve
8) Shut off main power

Table 2 Experiment condition

Exp. No.	Sample	Amount to use
Flight 1	1.AB-solution 2.AB-vessels	200 ml 10 tubes
Flight 2	1.AB-solution 2.AB-vessels	200 ml 10 tubes

For each flight, five same experiment cycles were done. For each experiment cycle, motor push two syringes.

Result and discussion

1. **Microgravity**

1st flight:

The experiment was running well during microgravity and preparation stage. No problem with mechanical parts. Some leaking problem was found in tube number three but this was not effect to the experiment of another tube samples.

2nd flight:

Leaking problem has been fixed but problem of mechanical parts was found. The two experiments were run automatically before microgravity stage. The 1st experiment was running during normal gravity (preparation stage) and the 4th experiment was running during hyper-gravity.

From the 1st and 2nd parabolic flight we have got 16 samples from experiment during microgravity, 2 samples from normal gravity and 2 samples from hyper gravity.

Fig.10 Experiment Equipment

2. On ground analysis

Cholesterol concentration of input AB-solution (before flowing through scaffold tube) and output AB-solution (after flowing through scaffold tube) was measured by mixing with cholesterol test kit solution (Cholesterol liquicolor Human GmbH) and measured absorbance at wavelength 500 nm, visible light.

From the calibration curve, absorbance was converted to concentration and amount of cholesterol of each sample can be calculated by the equation $Y=0.00155*X$, $R^2 = 0.99$.

Table 3 Absorbance of AB-solution with Cholesterol liquicolor at 500 nm of experiment on ground experiment

Exp. No.	**Absorbance of input AB-solution**	**Absorbance of output AB-solution**	
		1st experiment	**2nd experiment**
1	0.282	0.262	0.269
2		0.263	0.256
3		0.258	0.258
4		0.259	0.263
5		0.259	0.259
6		0.260	0.257
7		0.262	0.266
8		0.261	0.256
9		0.259	0.257
10		0.258	0.256

Table 4 Absorbance of AB-solution with Cholesterol liquicolor at 500 nm of experiment on board

Number of sample	Absorbance of input AB-solution	Absorbance of output AB-solution	
		1st flight experiment	2nd flight experiment
1	0.282	0.145	-
2		0.194	-
3		0.193	0.184
4		0.191	0.166
5		0.145	0.160
6		0.156	0.182
7		0.133	-
8		0.147	-
9		0.161	0.174
10		0.149	0.144

Table 5 Summary of input-output AB solution, cholesterol concentration, amount of cholesterol per sample and Amount of missing cholesterol.

Experiment condition	input AB-solution			output AB-solution			Amount of missing cholesterol (mg/samples)
	Average absorbance	cholesterol concentration (mg/L)	amount of cholesterol (mg/sample)	Average absorbance	cholesterol concentration (mg/L)	amount of cholesterol (mg/sample)	
On ground	0.282	188	3.76	0.260	168	3.36	0.40
On board				0.165	106	2.12	1.64

From Table 5, indicated that the different gravitational condition affects to the amount of missing cholesterol of output AB-solution which was assumed to represent the amount of accumulation on AB-vessels inner surface. The average amount of missing

cholesterol of on ground experiment is 0.40 mg/sample, on board experiment is 1.64 mg/sample.

Under normal gravity condition, the cholesterol particles move from the syringe down into the blood bags in certain directions because of fluid's flow direction together with the gravity force. Under microgravity condition, the cholesterol particles move from the syringe down into the blood bags more random direction. It represents that **under microgravity condition, the cholesterol particles have more probability moving toward the vessel's wall and lead to the more accumulation.**

Conclusion and further perspectives

The result proves that gravity significantly affects the surface accumulation of cholesterol flowing in a vessel like coronary arteries. **Under microgravity condition have more amount of missing cholesterol of output AB-solution than under normal condition (Fig.11). This result indicated that under microgravity condition, cholesterol has more tendency to accumulate on the vessel's wall than under normal gravity condition.**

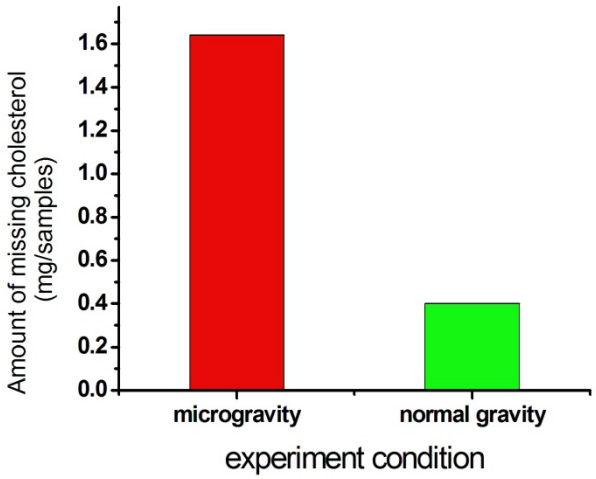


Fig.11 The amount of missing cholesterol of output AB-solution under micro gravity and normal gravity

For the further development, more experiment condition should be conduct to define other parameters that effect to the accumulation under microgravity, such as flow's direction and flow's pattern. The property of material and mechanical property which more similar to human body is also need, in order to get more accurate data that can be used to develop the solution to cure the cholesterol accumulation under microgravity for astronauts in the future.

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