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Automation of the Coronary Artery Preparation Process for Atherosclerosis Studies

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Abstract. This project proposes the development of a system for the improvement of gelatin injection in coronary arteries at the School of Medicine of the University of São Paulo (FMUSP). The system consists of an automatic injection device and a temperature controller - to keep the gelatin at a constant and adequate temperature. The coronary arteries are filled with gelatin to prevent collapse that would make the analysis process, that is performed after the dissection of the arteries of the heart, impractical. The device proposed here is a 'syringe pump', a microprocessed medical device which operates by means of a step motor coupled to a spindle, which propels the plunger of the syringe the temperature controller will take advantage of a Becker electric heater from the FMUSP research laboratory and will switch the device based on the temperature of the gelatin, captured through a temperature sensor. The expected result of the research is the successful development of these devices, which will increase effectiveness of the procedure, reducing the time and wastage of material in the process and maintaining a quality standard for conducting the atherosclerosis research.

Keywords. *Atherosclerosis, Coronary Artery, Medical Instrumentation, Automatic Injection.*

Introduction. Ischemic heart diseases, mainly associated with atherosclerosis, are the main causes of death in the state of São Paulo according to data from the State Department of Health, 2013 (1) with about 75 deaths per day. Thus, research associating atherosclerosis with other risk factors is extremely important in identifying patients long enough to treat and reduce the number of deaths.

FMUSP has several researches on ischemic heart diseases, mainly about atherosclerosis; one of these studies stands out for quantifying different measures of adiposity and atherosclerotic severity in autopsies performed on many subjects with different ages and social classes. The research will help to identify which of these measures is more associated with the systemic atherosclerosis, allowing to reach individuals with greater risk of disease and cardiovascular events.

To identify the degree atherosclerosis severity in the coronary arteries of the human heart, we need to prevent artery collapse filling the arteries with gelatin; if not, the image analysis afterwards could give false results or become damaged by collapse. The process is time

consuming and to increase the number of autopsies and tissue collection, the development of the syringe pump equipment and the gelatin temperature controller meet these needs. These devices will increase the gelatin injection speed and the effectiveness of the procedure.

Literature Review. *Coronary Arteries.* In the human body, the whole heart and blood vessels constitute the cardiovascular or circulatory system, which is responsible for providing the transport of oxygen, nutrients and hormones to the various systems and tissues. Within this system are the coronary arteries that are responsible for supplying the heart of blood; the heart, like any muscle, needs highly oxygenated blood to operate normally (2).

These arteries are divided into small arterioles that externally surround the heart and irrigate it with blood. The significant importance of the coronary arteries is to provide blood to the cardiovascular tissue (heart), so any disease or damage can have serious implications reducing the flow of oxygen and nutrients to the heart (3). Atherosclerosis is the main cardiovascular disease, classified as coronary disease because it disturbs this artery.

Atherosclerosis. Coronary artery disease is defined as 'blockage or narrowing of the coronary arteries' (2) and its main cause is atherosclerosis. According to Libby P. (4) atherosclerosis is characterized by the formation of atheroma (fat plaques) causing the stiffening of the walls of the coronary arteries; this is a progressive disease characterized by the asymmetric thickening of the inner layer of the coronary.

In the coronary arteries, the formation of atherosclerosis begins through a vascular endothelial injury - artery wall - due to several factors, the main ones being modifiable smoking, systemic arterial hypertension, diabetes mellitus, renal disease and adiposity (5). This lesion ends up forming an inflammation that causes lipoproteins to be retained within the subendothelial space. This process, coupled with the recruitment of lymphocytes (white blood cells) into this space, results in layers of cholesterol that accumulate irregularly in the lining of the artery. The development of this layer of cholesterol leads to loss of elasticity of the artery and narrowing of the lumen, which is called the internal arterial cavity, making it difficult for blood to pass through the vascular tissue.

The process progresses with the accumulation of calcium deposits on the fat plate, the so-called calcification process, and leads to weakening and possible plaque rupture. When the atheroma ruptures, it can lead to an even greater reduction of the lumen, with blood entering the ruptured atheroma, and the formation of a blood clot, the so-called thrombus, detaching from the artery and moving through the blood to an artery of smaller caliber leading to its possible occlusion.

Libby, Ridker, and Maseri (6) define atherosclerosis as an ongoing inflammatory response and, according to advances in science, inflammation plays a key role mediating all stages of onset progression leading ultimately to thrombotic complications of atherosclerosis. Still second authors, these relationships provided important insights between risk factors and the mechanisms of atherogenesis.

Atherosclerosis plaques - called atherosclerotic plaques - can be classified into two types: stable and unstable. According to Nishizawa (7), stable plaques are characterized by collagen

dominance, organized in a thick fibrous cap, whereas the unstable plaques have an intense inflammatory activity and a thin layer. The rupture of the plaque exposes a highly thrombogenic lipid material - which can produce a thrombus or which can easily clot the blood. This process is called atherothrombosis and is one of the main clinical manifestations of atherosclerosis.

The consequences of atherosclerosis are diverse and can affect not only the cardiovascular system but also others; related diseases include ischemic disease (including acute myocardial infarction, unstable angina and sudden cardiac death) of the heart, peripheral arterial disease and aortic aneurysms (7). Atherosclerotic plaque rupture forms the thrombus, as mentioned previously, and this thrombus, traveling through the vascular tissue can cause a stroke, it is estimated that 75% of these accidents have thrombotic origin (8).

Methods. *FMUSP Research.*

(a) Association between adiposity and systemic atherosclerosis. The method research is composed of analysis of different samples of deaths over 30 years or more; sociodemographic information and risk factors for cardiovascular diseases will be analyzed. To make measurements on the fat will be measured the neck circumference, waist and hip, also weight, height and thickness of abdominal subcutaneous tissue - in this way will be calculated BMI, waist-hip ratio, hip-height ratio and index of body-shape. The weight of the pericardial and abdominal fat, the heart and the left ventricular wall thickness will also be measured. The presence of myocardial infarction, the degree of atherosclerosis of the aorta, carotid, coronary and cerebral arteries and plaque composition in the same arteries will be evaluated.

(b) Analysis and Evaluation of Systemic Atherosclerosis in Coronary. First, the heart of death must be donated to the laboratory through family donation to science. When the heart is donated it is first received by the section of pathology that oversees analyzing the causa mortis. In this section, the heart receives a cross section so that the presence of infarction in the myocardium is analyzed, this cut, in most cases, causes a division of the coronary artery into two parts. Then the heart is sent to the FMUSP laboratory; the assessment of systemic atherosclerosis is performed mainly through image analysis, the collapse of the artery walls makes this process impossible, so filling of gelatin inside the arteries occurs. The injection procedure is all done manually with a 20-mL syringe: first, injection of hot gelatin occurs directly into the received heart (which is cold for tissue preservation), then the heart is taken to the freezer, at a temperature at -20º C for 5 minutes, so the gelatin solidifies. The process of making the gelatin, in the laboratory, is done without temperature standards, the water is boiled and, after three to five minutes the powders is mixed with the water and stirred. The coronary arteries are then dissected from the heart, in this stage, injections of hot gelatin are done again into the already dissected arteries. This happens because the first injections done into the not dissected coronary arteries is insufficient for filling completely the arteries. This last step leads to formaldehyde fixation for posterior image analysis and evaluation of atherosclerosis.

Conceptual Project. The development of this work is for the development and implantation of two devices: a semi-automatic gelatin injection system and a gelatin temperature control. That is needed because the various injections of gelatin that occur in the coronary arteries increase the procedure time and may lead to the impossibility of the process of posterior image analysis.

The manual way the injections are placed can further damage the artery walls by overpressure of the injection and cause damage to the user due to repetitive strain injury; the temperature control can also damage enzymes or proteins of the plates, in the current procedure there is no measurement or temperature control of the gelatin. Also, the FMUSP research aims to collect 500,000 subjects in five years, this project could improve the procedure and diminish the time making that aim more tangible, preventing lesion from the doctors.

Results. To develop the concepts, sketches and ideas the authors monitored and performed the procedure taking notes and making observations and comments with the doctors. This helped to encompass all aspects of the procedure, with potential problems or implementations with the solution. One of the possible changes suggested was the change of fluid injected in the procedure, instead of using only gelatin, use mixture of formalin and gelatin.

This was suggested to improve the effectiveness of the first injection in the procedure, making subsequent injections unnecessary, also decreasing the total time of the procedure. Gelatin has its physical characteristics due to its ability to form matrices with high immobilization yield (9). These continuous and cohesive matrices can be formed from protein-protein or protein-other clusters depending on their iterations with other components (10). Reactions between formaldehyde and gelatin at neutral pH (6 to 7) significantly increase molecular weight, viscosity, and decrease water absorption capacity (11). Experiments conducted by R.A. de Carvalho and C. R. F Grosso (10) have shown that a mixture of formaldehyde with gelatin causes an increase in tensile strength and consequent strength as a function of the formaldehyde concentration. Testing different concentrations of the mixture in different coronary arteries the optimal fluid result was the mixture 35% formaldehyde at 38 degrees Celsius. The test also revealed that small cuts at the end of each coronary help the injection procedure forming an escape route for air in the arteries.

Syringe Pump. To perform automatic injection, after some research, the authors chose the ‘syringe pump’ design: has relative simplicity of operation, the conversion of electric energy into motor mechanics allows the rotor to generate angular momentum and angular displacement, through the coupling with the linear spindle occurs the transformation of this moment and angular displacement into linear. With a fixed flange on a movable platform where the syringe is attached (Figure 1), the motor presses the plunger of the syringe allowing the injection to be performed at constant speed, given the control settings of the microcontroller. The microcontroller chosen to this project was the Arduino Nano, due to its facility of use and programming and the possibility of connecting different shields such as the LCD shield. The stepper motor chosen was a Nema 17 with spindle, this saved time and effort due to this built-in

mechanical attachment, the stepper motor driver carrier chosen was the A4988 which features adjustable current limiting, over-current and over-temperature protection and five different microstep resolutions (down to 1/16).

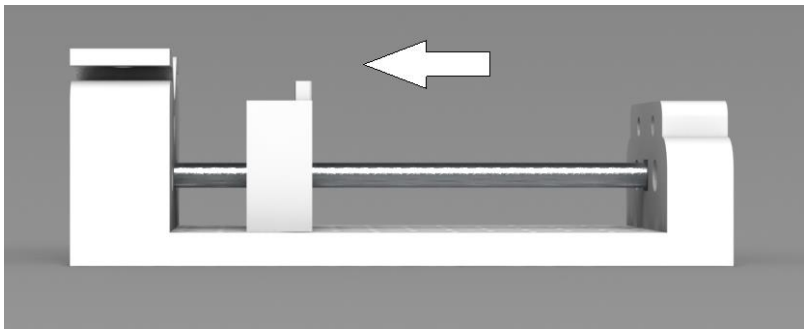

Figure 1. Operation Principle – Syringe Pump

In order to test the concept of the syringe pump, and modify the design to accommodate all requirements a 3D model was built on the software SOLIDWORKS (Figure 2). This design was approved by the FMUSP laboratory team to begin prototyping.

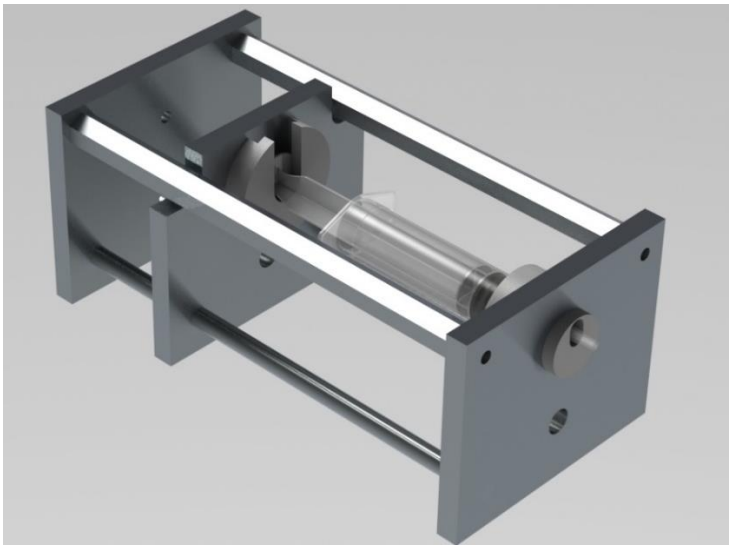

Figure 2. Model of the Syringe Pump developed in SOLIDWORKS

The prototype was built in Aluminum, with some machined polyamide pieces to facilitate the fitting of the syringe, all dimensions respect the size of a 20-mL syringe, mainly used on this procedure, although using 10-mL syringes could also be used. The prototype is shown on Figure 3.

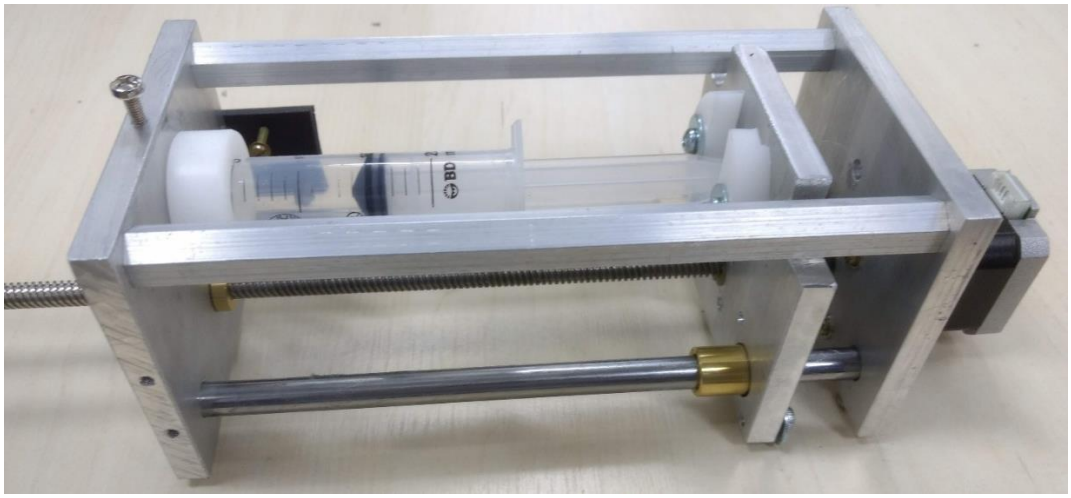

Figure 3. Built prototype

There is also the implementation of a new protocol based on the program flowchart, developed for using the syringe pump. This will optimize the process using the full potential of the device. First the system starts with the referencing routine, that makes use of one of the end-of-course keys, to position the platform to start the process. The user then inserts the syringe on the device, firmly securing it, and presses a button to start the injection. To improve the safety of the procedure the injection only takes place when the button is pressed, this ensures that the filling of the arteries is done only when needed. When the platform activates the second end-of-course key, placed at the of the device, the injection breaks and stops. The user can reset the device, pressing another button. Some parameters of the injection procedure can be configured: velocity and acceleration; there is also a jog mode implemented, where the user can control the displacement of the mobile platform, placing it where is needed. The program flowchart is showed below:

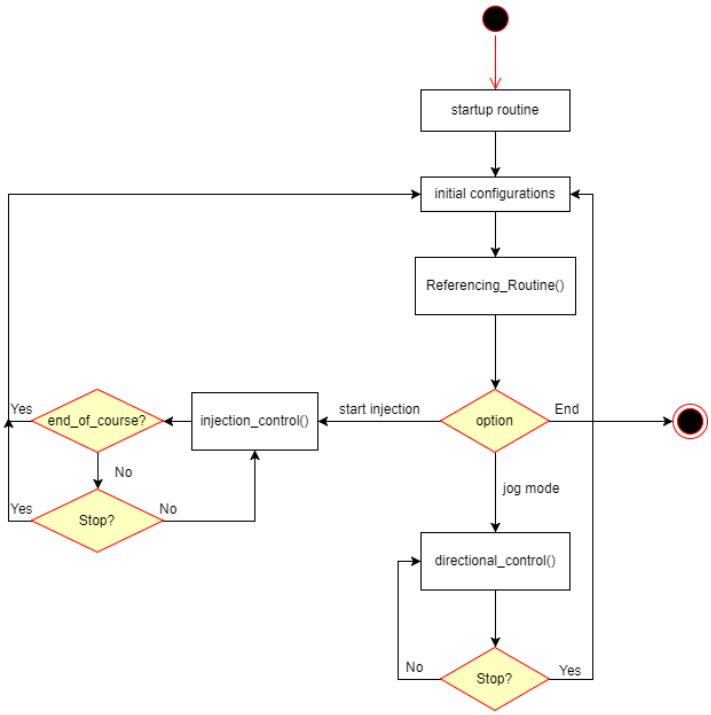

Figure 4. Program flowchart

Temperature Controller – Thermostat. In the FMUSP laboratory, the heating of fluids - the water for the gelatin powder mixture and to keep the gelatin warm – is done on a Becker electric heater; this device only has control of the heating power and does not provide display of the current temperature, control of the temperature or setpoint of temperature. The lack of these features can result in a non-standardization of the process and could decrease effectiveness and performance of the procedure.

In order to develop a device that would take advantage of this equipment, already available in the FMUSP laboratory, and that would not modify its technical features, a thermostat was suggested. This device works using a temperature sensor as feedback, comparing it with the setpoint temperature set by the user, to switch the state of a power relay, connected to an outlet where the Becker electric heater power outlet can be connected.

The device chosen was the W1209 board, a thermostat module with sensor input, keys, display and relay. The temperature sensor chosen a NTC (10 K 0.5%) thermistor is waterproof and because of that can be used for this application. One of the advantages of using this board are the different available settings such as: hysteresis, temperature correction, time delay, alarm, among others. The most important feature of this device is that, unlike the syringe pump that is dedicated to this research, it can be used in several different research procedures in the laboratory; it is only necessary to heat or control the temperature of a fluid.

Conclusion. The successful syringe pump prototype proved the concept of the proposed solution: the device can perform injection and the design chosen can improve the gelatin injection procedure. This device is currently being perfected, as some minor adjustments were necessary. The addition of formaldehyde to the gelatin solution already proved itself a considerable improvement, as it was share by FMUSP staff, already reducing the number of injections needed to completely fill the coronary arteries. The temperature controller prototype is currently being built for testing, but already proved itself needed by the FMUSP staff because of its versatility of use in other procedures and laboratory researches. In the future, it is desired do develop a closed loop control in the syringe pump device with a piezometric pressure sensor; also, to build an electric heater with temperature controls built-in.

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