Measurement system for an in-vitro characterization of the biomechanics and hemodynamics of arterial bifurcations

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Abstract. Arterial behaviour in-vivo is influenced, amongst other factors, by the interaction between blood flow and the arterial wall endothelium, and the biomechanical properties of the arterial wall. This interaction plays an important role in pathogenic mechanisms of cardiovascular diseases such as atherosclerosis and arteriosclerosis. To quantify these interactions both from biomechanical and hemodynamical standpoints, a complete characterization and modelling of the arterial wall, blood flow, shear wall and circumferential wall stresses are needed. The development of a new multi-parameter measurement system (distances, pressures, flows, velocity profiles, temperature, viscosity) for an in-vitro characterization of the biomechanics and hemodynamics in arterial bifurcations (specially in carotid bifurcations) is described. This set-up represents an improvement relative to previous set-ups developed by the group FCIEN-FMED and is presently under development. Main subsystems interactions and environment-system interactions were identified and compensated to improve system’s performance. Several interesting problems related with signal acquisition using a variety of sensors and some experimental results are shown and briefly discussed. Experimental data allow construction of meshes and parameter estimation of the biomechanical properties of the arterial wall, as well as boundary conditions, all suitable to be employed in CFD and FSI numerical simulation.

1. Introduction
Arterial behavior in-vivo is influenced, amongst other factors, by the interaction between blood flow and the arterial wall endothelium, and the biomechanical properties of the arterial wall. This interaction plays an important role in pathogenic mechanisms of cardiovascular diseases such as atherosclerosis and arteriosclerosis. To quantify these interactions both from biomechanical and hemodynamical standpoints, a complete characterization and modeling of the arterial wall, blood flow, shear wall and circumferential wall stresses are needed. Characterization can be done in-vivo and in-

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vitro. In-vivo, by means of invasive and non-invasive (tonometry, pulsed Doppler and pulse wave velocity) methods. In invasive methods, suitable instrumented specimens are used to perform the measurements on a given arterial segment. The specimen is selected depending of the objectives of the experimental work. In-vitro, the characterization can be done by means of a hemodynamic workbench simulator (HWBS) in which arterial segments are intercalated in closed fluid circuits that emulates the main features of systemic arterial circulation [1-3]. A sound in-vitro characterization of arterial segments (including pressure and flow patterns) may furnish valuable information to be applied in-vivo [4]. A specific experimental set-up is needed in order to be able to work with arterial bifurcations. Measurements obtained from cryopreserved [5] and fresh arterial bifurcation samples (for example carotid bifurcation amongst others) subjected to different hemodynamic regimes allow the characterization of the biomechanical properties of arterial segments. These are obtained by parameter estimation and will be employed in a model for the numerical simulation of the interaction between structure and fluid. Velocity profiles obtained in the workbench will be compared with the ones obtained by CFD simulations with moving wall (figure 1).

Figure 1. In-silico characterization by FSI+CFD. Coupling of 3D, 1D, and 0D models to perform a fluid-structure interaction analysis of the flow and wall motion in an arterial bifurcation.

The main purpose of the present paper is to describe the development of a new multi-parameter measurement system for an in-vitro characterization of the biomechanics and hemodynamics in arterial bifurcations. This set-up represents an improvement relative to previous set-ups developed by the group FCIEN-FMED and is presently under development. It poses several interesting problems related with signal acquisition using a variety of sensors. Some representative experimental results are shown and briefly discussed.

2. Hemodynamic work bench simulator (HWBS)
The HBWS closed circuit is composed by a pump, the tubing between the pump exit and the entrance to the arterial bifurcation, the arterial bifurcation being studied, tubing between each branch of the bifurcation exit and the entrance to a fluid reservoir, adjustable constrictions on each branch, a fluid reservoir with mean pressure adjustment, and a returning tubing from the reservoir to the pump (figure 2).

Figure 2. HWBS fluid circuit schematic diagram. Compliant silicone tubing is used for the circuit.
An artificial heart (Cardiobot) is used as the pulsatile pump, with frequency and ejection volume adjusted at demand. Cardiobot is an electronically controlled programmable pump, specially developed at LAU-FCIEN-UdelaR [6] in order to generate different flow and pressure patterns in the HWBS. Cardiobot generates a trigger output at the beginning of each systole. This signal is used as a time reference and for triggering.

The output load impedance distal to each branch of the arterial bifurcation can be adjusted by means of variable restrictions (variable clamp). For each branch, there is an independent restriction located far away from the corresponding branch output. Each restriction introduces localized mechanical impedance mismatch that produces reflected waves. The combination of upstream and downstream pressure pulses produce global pressure patterns in the arterial sample. Adjusting each restriction (amongst others bench parameters), near-physiological pressure pulse and flow patterns can be obtained in the bifurcation.

In each experimental configuration, the artificial heart is operated at constant frequency. The measurements are done after a steady regime of pulsatile flow is attained in the circuit. The already established dynamic regime and the trigger signal allow relocation and exchange of sensors during measurements (for example, A-Scan and Pulsed Doppler measurements can be done in the same region alternating probe placement). The samples are mounted on an arterial bifurcation fixing system (couplers and adjustable arms) and submerged in a physiological solution pool. This pool is rounded by an external water pool where temperature control can be done (figure 3).

The circuit can be filled with physiological solution or blood treated with EDTA sodic (rheological properties previously obtained with Brookfield, LVDT –II+ plate-cone viscometer). Temperature control can be done in the reservoir and in the external pool, with a set-up point of 37°C (measured using one thermistor in each of the above mentioned recipients). The reservoir is a glass flask containing blood or physiological solution and air. It's mean pressure can be adjusted at constant values by means of a hand pump and a differential pressure sensor. The HWBS can be operated with arterial samples, phantoms or other man-made tubes or bifurcations under static and dynamic conditions.

Other experimental setups are designed and operated exclusively to characterize mechanical properties of the arterial or venous walls, but can't be used to study wave propagation and fluid-structure interactions [7]. Bertram et al. [8] developed an experimental setup for arterial or tube segments that allow pulse propagation measurements under near physiological pressure pulse and flow patterns. Their setup employs rigid wall tubing. Two closed fluid compliance reservoirs (with a pressurized air chamber inside) are located before and after the sample. The first one attenuates
pressure transients originated in the closure of the valves of the piston pump. The second one is intended to allow the modification of mechanical impedance. A Darcy type hydraulic restriction located after the second chamber introduces a linear loss of charge. However it can’t be adjusted during operation. Our setup allows the adjustment of the two restrictions independently and during operation.

3. Description an discussion of system components and their interactions
The sample is positioned in the bifurcation fixing system trying to maintain the original geometry of the sample (lengths and angles measured during sample extraction surgery). Then, we fill the circuit with the fluid and purge the bubbles. With no flow and no transmural pressure in the circuit, measurements are done over the bifurcations in A-Scan mode in order to obtain lengths, internal and external diameters and wall thickness (morphometric data useful to construct a simplified finite-element grid for a CFD model). A 15MHz Panametrics V313 probe fixed on a mechanical graduated (1.5mm resolution) positioning system is connected to a Lecoeur A-Scan module controlled by a PC running correlation algorithm developed to obtain the measurements (figure 4).

After that, several hemodynamic conditions are simulated and measured (figure 5), adjusting the flow pattern of the Cardiobot, reservoir pressure and the variable restrictions (each one far enough from the output of each bifurcation branch, in order to obtain near-physiological flow and pressure patterns in the sample). The non-linear characteristics of this kind of restrictions are similar to those imposed by cardiac valve stenoses [9]. External diameter measurements are taken at suitable points in each segment of the arterial bifurcation using a gold-standard System 6 Mainframe equipped with sonomicrometer ultrasonic modules [9] and ultra resolution techniques [4]. Sonomicrometer ultrasonic crystals (5 MHz, 2 and 3mm diameter) were handcrafted and fixed to Dacron patches. These crystals are sutured to the outer face of the arterial wall (adventitia) in opposite positions relative to a diameter. Pressure waveforms are obtained using Königsberg sensors P2.5-S with high frequency response (1kHz) located near diameter measurement points. Sensors are sutured sensing surface points to the lumen in order to obtain the hydrostatic component of the pressure. Velocity profiles at different locations are obtained using a multigate Doppler system (DOP, Signal Processing SA). Micro-spheres powder (scatterers) are used when the HWBS circuit is filled with a physiological solution or water-glycerine mixture fluid. Cross sectional flow is measured using suitable diameter perivascular transit time flowmeters (Triton flowmeters).

![Figure 5. Schematic diagram of sensor placement.](image)

A Statham pressure sensor (located in the tubing near the common segment) is used for monitoring and initial adjustments procedures. In order to give a time reference for the measurements of the different sensors a periodic rectangular pulse signal is generated by the Cardiobot related with pulsatile pump function. Signals are acquired at 3kHz using 12-bit data acquisition modules (NI USB-6009 and LabJack U3-HV) operated from a specially developed Matlab application. Two Tektronix TDS2024B,
100MHz-4 channel digital oscilloscopes are used for initial adjustments and for monitoring purposes. After all the sensors are placed (and after temperature stabilization), gain and offset adjustment of modules is done. Voltage vs. diameter and voltage vs. pressure curves are obtained (pressure sensors are calibrated against a mercury manometer with static measurements at discrete pressure values). Estimation of propagation velocity of sound in the sample wall and wall thickness can be done using an auxiliary acoustic mirror located at a known distance from the Panametrics probe and with the sample between them. These parameters are loaded in the A-Scan developed processing software. Pressure and diameter samples are processed on-line in a computer running a specific developed algorithm. Biomechanical properties of the wall are obtained by means of model parameter adjustment [10].

The generalized static and dynamic characteristics of the sensors [11] used in the new system were checked. To validate the experimental results obtained using the new multi-parameter measurement system, we studied the performance and interoperability of each sensor in the framework of the whole measurement system, taking into account possible external influences. A survey of possible external influences was done. The influence of noises of the same physical nature as the input signals to the sensors and the EMC aspects were studied. For example, sonomicrometer measurements are sensitive to external radiated RF perturbations. These perturbations generated spurious triggering at the RF processing stage, altering the stability of the measured distances. This was corrected by the use of special shielded cable arrangements. Other radiated and conducted electromagnetic interference was reduced by the implementation of a shield to isolate the workbench from its external environment.

Table 1 shows the main interfering and modifying inputs and their possible compensation, both between environment and HWBS system and between HWBS subsystems. As an example of interfering input, let us consider transit time flow measurement. Also, let us consider that transit time flowmeter internal diameter is slightly bigger that external arterial diameter. Pulsatile flow causes vessel diameter variation at the cross sectional flow measurement points. This diameter variation is an interfering input for the flow meter. Transit time flow meter calculates cross sectional flow $Q(t)$ by measuring upstream $T_{upstr}$ and downstream $T_{downstr}$ propagation times [11,12]. The voltage output $V_{out}$ depends on the time difference, system gain $\chi$ and an error $\delta T$:

$$
V_{out} = \chi (T_{upstr} - T_{downstr}) + \chi \delta T
$$

$\delta T$ is mainly of electronic origin and $\chi \delta T$ can be at least partially compensated. The time difference verifies:

$$
(T_{upstr} - T_{downstr}) = K \cdot \left( \frac{Q(t)}{R_i(t)} \right)
$$

$K$ depends of internal parameters of the module, speed of sound and nature of flow (laminar or turbulent). So, neglecting $\delta T$, $V_{out}$ is proportional to $\frac{1}{R_i(t)}$ and $Q(t)$. The internal radius of the vessel $R_i(t)$ is an interfering input that modulates the flow signal. In general, $\left| \frac{\delta R(t)}{R_i} \right| \leq 0.1$, so an error of up to $\pm 10\%$ can be expected in flow measurement. This error can be compensated in post processing if internal diameter is measured at the same region and same time (assuming a circular cross section vessel). As an example of modifying inputs, let us consider A-Scan measurement. Environmental and laboratory EMC interferences are modifying inputs that can be compensated by the nature of the processing algorithms (averaging and cross correlation of echo signals). Other sources of interfering and modifying inputs and their possible compensation techniques can be found in table 1.

Before operating the system with arterial samples, suitable phantom models where used for sensor calibration and error estimation. The biomechanical properties of the arterial segment obtained by parameter estimation will be employed in the model for the numerical simulation of the interaction.
between structure and fluid. Velocity profiles obtained in the workbench will be compared with the ones obtained by CFD simulations with moving wall.

### Table 1. Main interfering and modifying inputs and their possible compensation.

| Subsystem       | Main Interfering input | Possible Compensation techniques                                                                 | Main Modifying input                              | Possible Compensation techniques                  |
|-----------------|------------------------|---------------------------------------------------------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Sonomicrometer  | US waves of other subsystems | Spatial disposition of subsystem sensors                                                        | Cardiobot power stage interferences              | Special coaxial cable configuration for receiver. Sonomicrometer trigger level adjustment |
| Sonomicrometer  | US waves of other subsystems | Non-simultaneous operation (only for nontransient operation)                                     | Other laboratory and environmental electrical interferences | Metal wire mesh shielding, dedicated online double conversion UPS with isolation transformer. Sonomicrometer trigger level adjustment |
| A-Scan          | US waves of other subsystems | Spatial disposition of subsystem sensors                                                        | Cardiobot power stage interferences              | Echo Signal averaging. Cross correlation processing |
| A-Scan          | US waves of other subsystems | Non-simultaneous operation (only for nontransient operation)                                     | Other laboratory and environmental electrical interferences | Echo Signal averaging. Cross correlation processing |
| A-Scan          | Possible interfering spurious vibrations | Echo Signal averaging. Cross correlation processing                                                | -                                                | -                                                |
| Flow meter      | Variable vessel diameter in time | Opposing input (generated from simultaneous measurement of vessel diameter)                     | Other laboratory and environmental electrical interferences | Metal wire mesh shielding, dedicated online double conversion UPS with isolation transformer. |
| Flow meter      | Difference between sensor and vessel cross sectional area | Choose suitable sensor diam. or correction factor (if above opp input not applied)               | Internal and external noise                      | Output filter (100, 30, 10Hz)                     |
| Pressure (Sthatham) | Trapped air bubbles | Purging the catheter                                                                              | -                                                | -                                                |
| Pressure (Sthatham) | Mechanical vibrations/movement catheter | Short catheter. Avoid movements during measurements                                               | -                                                | -                                                |
| Pressure (Konigsberg) | Mechanical vibrations/movement catheter | Avoid tip shocks during measurements                                                               | -                                                | -                                                |
| Pressure (Konigsberg) | Temperature variations | Correction of measurements (known offset in mmHg/K)                                               | -                                                | -                                                |
| Pulsed Doppler  | Vessel movements (lateral) | Use transducer array (Open System Lecoeur)                                                        | -                                                | -                                                |

### 4. Examples of data obtained and post-processing

Static and dynamic measurements of diameters (external and internal), pressures, flows and velocity profiles were done in several locations in the common segment and branches of the bifurcations. Figure 6 shows the simultaneous acquisition of diameter, pressure and cross sectional flow (without filtering) for a location in the common segment as well as the trigger signal generated by Cardiobot pump (rising edge corresponds to systole beginning). Figure 7 shows velocity profiles (without filtering) obtained by pulsed Doppler, in the same place. Vessel pulsation can be seen through the variation of the profiles width over the x-axis (distance in mm).
Figure 6. Diameter, pressure and flow in the same location for a main branch. Trigger signal can be seen at the bottom.

Figure 7. Pulsed Doppler velocity profiles (mm/s) obtained for a main branch. Vessel pulsation can be seen through the variation of the profiles width over the x-axis (distance in mm).

Figure 8 shows diameter and wall thickness variation during a slow depressurization experiment suitable to estimate relaxed (quasi-static) elasticity modulus (figure 9). Measurements are obtained from A-Scan signals processed with a specially developed acquisition and correlation algorithm [5].

Figure 8. Internal and external diameters (measured with cross correlation techniques) vs. transmural pressure for a given cross section of the sample, during a slow depressurization experiment (from right to left).

Figure 9. Young modulus estimation for a given cross section of the sample.

5. Conclusions
Near-physiological flow and pressure patterns can be obtained for different kind of arterial bifurcations (specially for carotid bifurcations) by means of adjusting compliant tube lengths and hydraulic resistances as well as the Cardiobot pulsation patterns. This kind of flow and pressure patterns are not strictly necessary if the intention is a biomechanical characterization of the arterial wall. However, realistic flow and pressure patterns are necessary to study fluid-structure interactions in scenarios as near as possible to in-vivo ones.

Working in a steady pulsation regime with a time reference signal allowed us to take different physical measurements in the same point of the arterial sample locating there, at different time instants, different sensors and relating the signals recorded to the same phase of the periodic regime. This allowed us to overcome the difficulties due to the size of and interference between the different sensors. Of course, the duration of the whole experiment must be short enough so that the properties of the arterial bifurcation don't change significantly.
Although the static and dynamic characteristics of the sensors used were suitable for the attaining of the posed objectives, when the whole system was assembled, several modifying and interfering inputs appeared due to intra-system interference and system-environment interactions. Compensation techniques applied worked successfully.

The obtained measurements can be used for: 1-Mesh construction for CFD modeling using morphometric arterial sample data obtained by caliper measurements and ultrasonic measurements techniques (US probe fixed to the mechanical graduated positioning system shown in Fig.4). 2- Parameter estimation of the biomechanical properties of the arterial wall, suitable to be employed in a model for the numerical simulation of the interaction between structure and fluid. 3-Assessment of velocity profiles in order to make a comparison with the ones obtained by CFD simulations of the arterial carotid bifurcation sample, taking into account the movement of the arterial wall. Estimation of the endothelial wall shear stress for a carotid arterial bifurcation at different points, under a periodic pulsed flow can be done by CFD simulation. 4-Establish boundary conditions (pressure and cross sectional flow) at the entrance and at the exits of the arterial sample, for CFD-FSI numerical calculations.

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