Design of a Microsensor for the Measurement of Mechanical Tension from Resistance Blood Vessels

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Abstract

This work is focused on the development of a microsystem for the measurement of mechanical tension from resistance blood vessels. To date, the smallest vessels used for these tests are 100-120 µm in diameter, hence they do not belong to the resistance district. The current technique employs two wires which are manually inserted into the vessel. One of the wires is mechanically interfaced to a load cell. The proposed solution is the introduction of a purposely developed Silicon-based sensor. Aimed at defining the final specifications of the microsensor, first experimental tests have been performed with bigger vessels. Insulation and packaging issues have been faced for different experimental set-ups, and a preliminary methodology for a safe interaction with this biological system has been drawn out. With the proposed Silicon-based sensor, the minimum diameter of the microvessels could go down to 50 µm. The use of Silicon may also allow the integration in the same device of a chemically reactive coat for measuring concomitantly biologically active substances such as nitric oxide and carbon monoxide (dual mechanical/chemical sensor). Alternatives to the Silicon-based solution are currently under evaluation; they could represent a valid support to perform e.g. isotonic measurements and provide further relevant information to the clinicians.

Key words: micro-vessel, physiological measurements, strain-gauge sensor

1 Introduction

One of the main research lines active in the field of physiology consists nowadays of the study of the mechanisms which regulate the blood flow through the different organs. This control is mainly located in the small arteries which precede the network of capillaries and which, with suitable dimension adjustment, present different resistance to the blood flow (“resistance” blood vessels). Even though this is well-known, most of the recent studies have been performed by using, for methodological reasons, bigger blood vessels (“conduction” blood vessels), with results which can be not relevant or even incorrect. This work has taken inspiration from a by now well assessed method for the study of small blood vessels from fetal lamb lungs, published in 1992 Wang (1992). The pulmonary circulation undergoes major changes during the transition from the fetal to the neonatal state, resulting in a fall in vascular resistance. Through the years, the mechanisms responsible for the elevated resistance in the fetus and the dilatation of blood vessels at birth have been intensively investigated, and several vasoactive agents have been implicated in both processes. Uncertainties remain however about the nature of the involvement of different substances in transitional adjustments, since there is an incomplete knowledge of the factors conditioning the responses, and accordingly experimental findings cannot be translated into a precise functional scheme Wang (1994).

There are important repercussions in clinical practice, as regards new-born children that experience difficulty in breathing at birth. Nowadays is very important to acquire new knowledge and to find well assessed methodologies for performing experimental tests. Concerning in vitro tests, being the lamb a “big size” animal, these vessels allowed a manual set-up. A different situation occurs if the same tests have to be performed on the same or different vascular regions of small size animals, such as mice. In that case a completely new methodological approach would be needed, with both automation in the set-up of the preparation and augmented sensitivity in the signal recording system. The problem is quite relevant because leading physiology and physiopathology are performed mainly in the mouse, which is the only animal that allows experiments with targeted genetic mutations.

2 Current methodology: limits and application
The current set-up allows to measure the force generated in blood vessels with diameter of about 150µm and length of about 500µm. The microvessel is manually isolated by dissection in a physiological solution bath with a suitable oxygen concentration and then fixed (by inserting into the microvessel two 25µm tungsten wires) to a purposely devoted support. Afterwards, the preparation is moved to a second workbench and immersed in a physiological bath (Krebs Solution). A skilled operator performs all the procedure under microscope. The second workbench is equipped with two micromanipulators (M1 e M2), a force transducer (DSC-6, Perkin Elmer Instruments), a microscope and a temperature sensor for the bath (see Figure 1).

![Fig. 1. Current set-up](image)

The microvessel exerts in idling conditions a force which is recorded by the transducer; this force can increase and decrease depending on external agents which exert a direct action or modify endogenous principles for vasodilatation and vasoconstriction. The preparation set-up procedure, including dissection and fixing the vessel to the recording apparatus, requires the following time-schedule:
- 1 h for the dissection of the desired blood vessel;
- 2 h for the isolation of the blood vessel and the positioning within the measurement apparatus (including the wires insertion and their fixing to the supports).

The following steps, consisting of reading and recording the stresses when functional conditions change, go on for many hours, needed both for preparation steadying and for data acquiring.

During the set-up procedure the following phases are particularly delicate and may compromise the success of the whole experiment:
- insertion of the tungsten wires into the microvessel and their fixing to the support;
- transfer of the preparation into the bath equipped with the recording apparatus;
- pre-tensioning (pre-stressing) of the microvessel in order to set the initial stress (corresponding to the in vivo conditions) previously calculated.

Even if with some difficulties, this apparatus allowed to study in the fetal mouse the mechanisms responsible for the changes that occur in the pulmonary circulation at birth Wang (1995), Theis (1997), Theis (1998), Liu (2000), and also the regulation of a special blood vessel (Botallo ductus), big enough even in mouse Coceani (1999).

### 3 Development of a new sensing device

The proposed research consists both of the design and the fabrication of new microelectromechanical systems for the measurement and the recording of the force exerted by smaller “resistance” mouse blood vessels (diameter 50µm, length 50-100µm), and of the automation of some procedures now performed manually.

To this aim, the best way to start facing the problem, would have been the employment of “off-the-shelf” sensors, eventually customized with slight modifications. This solution has been implemented, and some knowledge about important issues has been acquired, as better explained in the following. However, the stated above commercial sensor needed a few structural modifications, and the overall procedure resulted to be highly not repeatable. Tests with a simpler system have been performed, with successful results. Moreover, as regards
future work, different principles are currently being investigated, and a short description of each of them will be provided in the following paragraph.

3.1 First steps

As a first step, a video-recording system for the documentation of the set-up phases of the preparation as they are now, has been introduced. The monitoring of the experiment progress by using a mini-CCD camera-based vision system allows not to have one single point of view (the stereomicroscopy of the operator), not suitable for the training of many operators. Further preliminary innovations, more related to the improvement of the overall system than to the sensing system, concern the introduction of motorized multi-d.o.f. micropositioners, like the ones (Marzhauser-Wetzlar - MS-314 model) now used at the CRIM Lab micromanipulation workstation Eisenberg (2001), Menciassi (2001), Carrozza (2000), Menciassi (2001).

Furthermore, a new mechanical design of the bath has been conceived (see Figure 2), in order to allow an easy use of the novel set of tools. Another objective was to minimize the volume of the bath, in order to have a reduced quantity of chemicals diluted in the solution.

As previously introduced, as preliminary work some tests with commercial Piezolevers from Thermomicroscopes have been performed. The piezolevers are piezoresistive cantilevers, and have overall size and thickness comparable to those of AFM traditional cantilevers (length: about 300 µm; width: 50 µm; thickness: 3 µm). They are for this reason really fragile, thus even if they are extremely sensitive (0.7x10^-6 [nN -1 ]) and in principle suitable for the proposed application, some structural modifications became necessary. They were strengthened by gluing a glass fibre (scheme depicted in Figure 3). Besides the strengthening function, the glass fibre had the key role of lengthening the part to be inserted into the microvessel. Preliminary tests were performed on vessels plastic simulators. They consisted of the insertion of the sensor (calibrated in force by using a load cell – Model GM2 3M, PTC Electronics Inc., Wyckoff, NJ, USA – full scale 300 mN, accuracy 0.01 mN) into the plastic small tube, in order to check the first steps of the procedure. The first part of the experimental procedure remained instead unchanged: after the manual dissection, the vessel was removed from the animal by using just one tungsten wire. The main advantage of the novel technique is that the employment of the second wire is by-passed, thus reducing sources of noise for the force transducer. The use of a piezoelectric nanometric traslator (PI, M-111.1DG, minimum incremental motion of 50 nm), mounted on the Marzhauser macromanipulator, allowed to easily control, by a PC graphic interface, the motion of the sensor during the approaching and the insertion phases. A drawing illustrates in Figure 4 the final arrangement.
Figures 5 and 6 show the sensor based on commercial piezoresistive cantilever and the results of the calibration, respectively. These tests, even if with some successful results, demonstrated that an off-the-shelf device is, for this problem, not the optimal solution. The sensors are indeed too tiny to be employed with no specific treatment, both in terms of mechanical strengthening and electrical insulation. Both procedures resulted to be time-consuming, not repeatable and definitely not safe as regarded the integrity of the sensor in its final configuration. It was decided therefore to perform the next tests by using a simpler configuration, i.e. a bigger commercial semiconductor strain-gauge sensor with a mouse aorta. This approach would have allowed to address relevant issues of the problem with an high repeatability, as better explained in the following.
3.2 A different approach

As stated above, the first trials performed with the Piezolever-based sensor represented an important starting point to the problem approach. Main attention was focused to the issues of sensor electrical insulation, packaging, sensitivity and robustness. As regards the material, Si has been devised, in most cases, as the elected choice, because of its mechanical properties and because of the microfabrication technologies which allow a strong miniaturization of the devices, e.g., Fauver (1998), Lin (2000), Miyazaki (2000). This miniaturization level will permit to carry out the above stated measurements on smaller vessels, which nowadays cannot be performed.

3.3 Experimental tests

In order to start facing in real terms the practical issues related to the experimental in vitro tests, further experiments on bigger vessels have been carried out. The employed sensor is a commercial semiconductor strain gauge (Entran, ESU-025; length: 1.27mm; width: 0.38mm), mounted on a rigid support as a cantilever (Figure 7 and 8).

The resistance variations are read by an external electronic circuit: the signal is lead to a Wheatstone bridge, whose output is amplified by a dedicated instrumentation amplifier (Analog Devices, AD620) with a variable gain. The supply voltage chosen was 10V, both for the Wheatstone bridge and for the amplifier, in order to have a wide dynamic range for the output signal.

The strain-gauge was electrically insulated with commercial coatings. It was difficult to achieve a proper insulation, also because during the first trials electrolysis phenomena occurred and part of the coating was dissolved in the saline bath, polluting the physiological conditions. In order to overcome this problem, some trials with different insulating materials were performed. The best results were obtained using a bio-compatible UV-curing epoxy adhesive (EL Electro-Lite Corp., Danbury CT, USA; ELC 4481). A reduction of electrolysis
phenomena could be achieved also reducing the Wheatstone bridge voltage supply, therefore decreasing the voltage at strain gauge terminals under the level needed from the electrolysis phenomena to start.

Static and dynamic force calibrations were performed using the system showed in Figure 9. It is composed by two micro-positioners holding the strain-gauge sensor and the nanometric translator, respectively. An high resolution load cell (Model GM2 3M) was mounted on the nanotranslator and orientated perpendicularly respect to the sensor. The static calibration was conducted by imposing a discrete displacement with step of 0.7 µm. The force and the resistance values reported in Figure 10 were acquired after some relaxation effects were expired.

Fig 9: Overview of strain gauge sensor calibration system

Fig 10: Static characterizations of strain-gauge sensor
Fig 11: Dynamic characterizations of strain-gauge sensor

For the dynamic calibration, a continuous motion (constant speed of 70µm/sec) of the load cell tip versus the strain gauge sensor was imposed. The results of this procedure are presented in Figure 11. The comparison between static and dynamic calibration forces points out the hysteresis effects that occur during the static measurements. However, even if the experimental data dispersion of static measurements is greater than those of the dynamic one ($R^2_{\text{static}} = 0.842$, $R^2_{\text{dynamic}} = 0.976$), the sensitivity values obtained by linear interpolations have the same order of magnitude ($S_{\text{static}} = 7.851e^{-7}$, $S_{\text{dynamic}} = 5.757e^{-7}$). This result confirms the correctness of dynamic measurement procedure respect to the static one.

The great stiffness of glass fibre used in the piezolever sensor and the low hysteric effects due to the absence of insulator material, ensure an higher linear behaviour of piezolever sensor than those of the strain-gauge one ($R^2_{\text{strain-gauge}} = 0.976$, $R^2_{\text{piezolever}} = 0.991$). Furthermore, the high precision technology employed to fabricate the sensible part of the piezolever device allows to reach a greater sensibility than strain-gauge ($S_{\text{strain-gauge}} = 5.757e^{-7}$, $S_{\text{piezolever}} = 1.101e^{-6}$). The drawbacks of the piezolever solution are, as stated above, the impossibility of a good electrical insulation and the fragility of the sensorized tip.

As the calibration process was concluded, in vitro tests on bigger vessels (freshly excited mice aortas: diameter of 700 µm and length of 2 mm) were performed. Figures 12 and 13 illustrate the overall system (with two manipulators, their control unit and the acquisition system) and the strain-gauge inserted into the vessel, respectively.

Fig 12: Overview of the measurement system (left); zoom of the bath area (right)
After the insertion of the sensor into the vessel (showed in Figure 13), in the heated bath (Krebs solution; temperature: 37°C; volume: 20 ml) Phenylephrine 10mM was dosed. This substance has the property to stimulate the receptors, so that the effect is a contraction of the vessel, in a lapse of time proportional to the endothelium integrity Chataigneau (1999).

During the test, the vessel started its contraction as shown in Figure 14. The variation of vessel force contraction in time is traced by recording the resistance variation of strain-gauge sensor and by using the calibration curve showed in Figure 10 and 11.

Both the transient time needed to reach a steady condition (about 6 – 7 minutes) and the final value of the force contraction (about 7.5mN) confirmed the effectiveness of the strain-gauge sensor technique.

The main problem of the strain gauge sensor developed lies in his dimensions, bigger than smallest microvessel that would be tested. In order to overcome this problem other solutions will be investigated in the meanwhile, as explained in the following part of the paper.

4 Future work: an overview

3.4 An alternative low cost sensor

An alternative low-cost solution could consist in fabricating SU-8 based sensors Thaysen (2002). SU-8 is a negative, epoxy based, chemically amplified resist. Figure 15 shows the main steps of this photolithography-based technique and a sensor which involves the employment of sputtered gold as piezoresistive material.
3.5 A Comb-shaped sensing system

Another proposed solution is a MEMS (Micro Electro Mechanical System) based on the balancing of the force due to the elastic behaviour of a mechanical structure, which adheres to the blood vessel specimen, and the electrostatic one, due to an array of interdigitated capacitors. This working principle is exploited in several accelerometers, gyroscopes and high selectivity electromechanical filters. The microsystem structure (Figure 16) is composed by a polysilicon suspended body, anchored to the substrate in four points. The proof mass includes a cantilever, which has to adhere to the blood vessel’s wall, and an elastic structure with 2 “comb drive” (array of interdigitated capacitors). In the frontal part of the substrate a dig will be realized with the aim of protect the extremely fragile cantilever and to allow an easy and safe insertion of the beam in the blood vessel.

Concerning the working principle, the blood vessel wall has to adhere to the cantilever: the specimen contraction will cause a lateral translation of the proof mass, so that a voltage difference is induced at the outer contact of the comb drive. Thanks to the electronic feedback, this perturbation can be balanced so that the cantilever could return in the starting position, because of the electrostatic force of the comb drive. Using this principle the only consequence of a vessel contraction would be a changing in the voltage output, and not a narrowing of the specimen lumen (isometric measurement). Of course the voltage output will be proportional to the strength of the blood vessel contraction.

3.6 A no-contact fluidic solution

The last principle is based on a no-contact measurement method (isotonic measurement) which avoids to use any sensor element in contact to the animal microvessel and to physiologic solution where it is bathed.
The sensor (see Figure 17) is composed by a microfabricated hydraulic path etched into structural substrate and closed by a thin lid. Furthermore, two piezoresistive sensors are located onto areas of lid covering the inlet and the outlet chambers of fluidic path.

The measurement procedure should be consist of to immerge the microvessel in the calibrated channel, to fix it onto ground with a wire, to close the device with sensorised lid and to enforce a steady fluid flow inside sensor. Being the fluid flow steady, for each changing in vessel diameter due to variations of activating specie concentration corresponds to a differential measurement of pressure sensors.

![Figure 17: Fluidic sensor](image.png)

3.7 Future plans

Further in vitro tests with freshly excised vessels will be performed exploiting the developed strain gauge sensor. Comparisons among first prototypes of sensors based on different working principles will be carried out.

5 Conclusions

In this work two different kinds of force sensor for microvessels contraction were implemented. The first, piezolever based, resulted to be very sensitive and linear, but also too much fragile to be covered with an insulating layer and used for in vitro tests. The second kind of sensor developed was based on a strain-gauge mounted in a cantilever configuration. Despite a lower sensitivity and an higher hysteresis, it gave good results during in vitro tests. The data acquired with the novel sensor well match the literature results. In the future works, the main goal to achieve is to design and to microfabricate a system able to accomplish both high sensitivity of piezolever and high robustness of strain-gauge sensors.

The project is interdisciplinary and intends to combine in a synergic way medical and engineering competencies. Moreover, there are good development perspectives, also commercial, of microsystems oriented to vascular biology. In fact, there isn’t now a method that allows to study these microvessels, and this project would pave the way for further research work, with important repercussions in the clinical field. Among pathologies which could be investigated, mainly relevant are pulmonary hypertension and dysfunction in the coronary and placentary circulation.

The use of Silicon may also allows the integration in the same device of a chemically reactive coat for measuring concomitantly biologically active substances such as nitric oxide and carbon monoxide (dual mechanical/chemical sensor): an interesting improvement could consist in integrating in the same microdevice different sensors, devoted to the measurement of both physical (such as forces exerted by the vessels during contraction) and chemical (such as the releasing of gasses like nitric oxide and carbon monoxide). The nitric oxide, in particular, represent an extremely important regulation factor for the vessels.

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References


Dear Arianna,

I acknowledge reception of your e-mail. Your paper will, as you wish, not be in the conference proceedings.

It was a very good paper and we regret your decision.

All the best,
:: Christof

> > Dear Mr. Teuscher,
> > we are very sorry to inform you that we have to withdraw the paper
> > submitted to IPCAT2003. The reason of this decision is related to non
> > disclosure problems which we have analysed in depth during the last
> > week. Please do not insert our paper in the Proceedings of the
> > conference.
> > We wish you a successful conference and we thank you for your
> > excellent management.
> > Best wishes.
> > The Authors

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