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REPORT

Effects of multidirectional vibrations on the microcirculation of the mouse skin

Part 1: Experimental protocol

Lymphology Research Unit
Supervisor: **Prof. Dr. Pierre LIEVENS**

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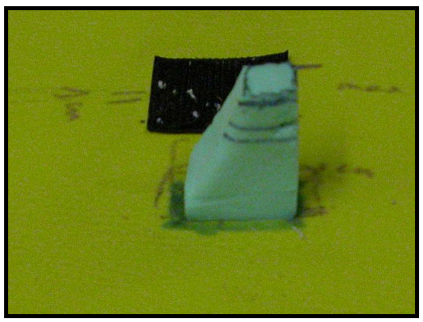
Purpose of the study

To determine if the multidirectional vibrations delivered by the "HHP Massage Mattress" have an influence on the blood and lymphatic microcirculation.

Materials and Methods

1/ Material

-**A small « HHP Massage Mattress »** especially designed by the firm of mattress, with exactly same characteristics as a truth « HHP Massage Mattress » (photography 1),



Photography 1:
Small HHP Massage Mattress.

-**An attachment system for the small mattress** (photography 1),

- **A binocular microscope :** ZEISS 50, West Germany, OpMi-1 89183
  - Ocular enlargement : 20 x,
  - Ojective enlargement : 6x, 10x, 16x, 25x, 40x,
  - An integrated source of light,

- **A digital SLR camera :** Nikon D 80,

- **A video-camera:** Sony DXC-101P,

- **A small dissection material,**

2/ In vivo lymphatic and blood vascular animal model

We used as lymphatic and blood vascular model, the lymph vessel of the lateral superficial epigastric vein (L.E.V.) of the mouse. This vessel connects inguinal to axillary lymph nodes (Fig 1). Beside the lymph and venous vessels, there is a small artery.

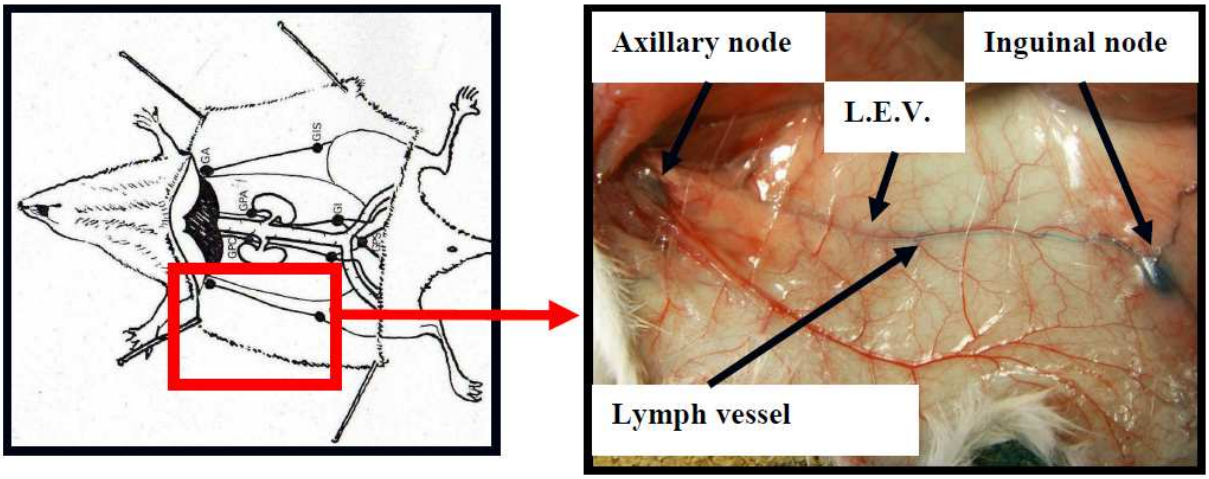


Figure 1: Inguinal lymph node, L.E.V., lymphatic vessel and axillary node after colouring with Evans Blue in the white mouse.

3/ Measurement of the vessel diameter

To determine if the multidirectional vibrations delivered by the "HHP Massage Mattress" have an influence on the blood and lymphatic microcirculation, we measured the diameter of the blood and lymphatic vessels on photographies taken under microscope during the experiments.

Analyzing method

We used the Image J software to analyze ours photographies.

Before the measurement of the diameters of vessels, we must very precisely know the enlargement of the photograph taken and parameterize the scales of measurements of the software.

We proceeded in the following way:

Once the camera (Nikon D80) installed on the microscope, we take for each objectives enlargements a photography of a squared blade (which each square made 1mm x1mm).

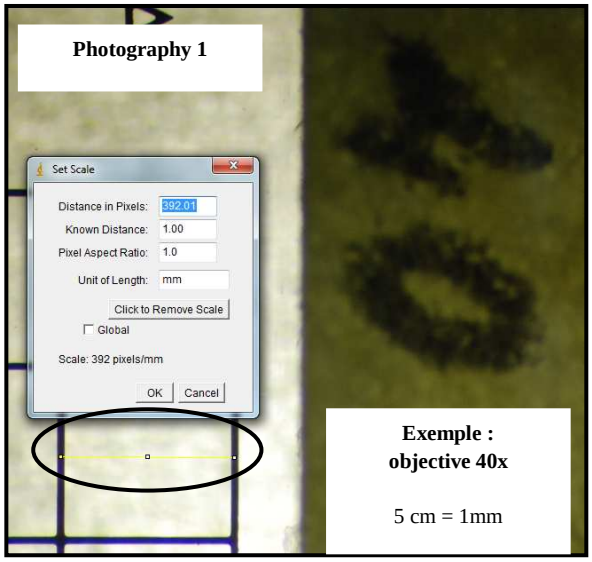
After, you open the picture with the Image J software:

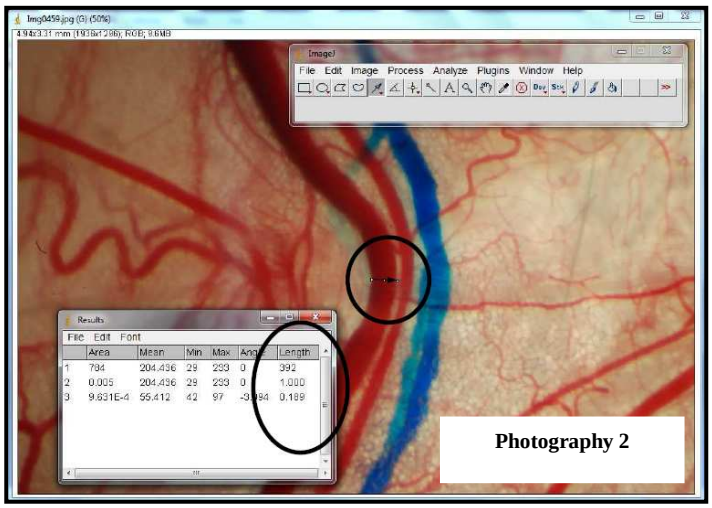
Size of photography : 1936x1296 pixels, RGB, 9,6 MB.

We measure in pixels the size of the square on photography, and we parameterize this distance (measured in pixel) in mm (photography 1).

For the other photographies taken with the same objective (x40), the software gives us the measurements in mm. Exemple photography 2: the reel diameter of the vein is 0.189 mm.

Scales	Enlargements : Microscope + Nikon D 80
0.9 cm = 1mm	6x
1.3 cm = 1mm	10x
2 cm = 1mm	16x
3.1 cm = 1mm	25x
5 cm = 1mm	40x





4/ Experimental protocol

We used 6 mice for this preliminary study. The animals used for our study are N.M.R.I. (Naval Medical Research Institute, Bethesda, Maryland, USA) female white mice, with an average weight varying from 28 to 30g and with an age ranging between 6 and 8 weeks.
Before anesthesia, animals rested for 20 minutes to acclimatize to the constant temperature in the laboratory (21-25°C). Animals were anaesthetized by means of a subcutaneous injection of urethane (with 25%). After abdominal skin shaving (with small scissors, not an electric mower which can distort the results), we proceeded to make a longitudinal incision along the Linea Alba and carefully dissected the abdominal skin. The shaved skin of the right half-abdomen of the mouse is inclined.
The direct injection of Evans Blue (to color the lymph vessels) was realized into the inguinal node (GODART, 1977; GEYSELS, 1990).

To examine the skin microcirculation by means of transillumination microscopy in vivo, each dissected mouse was placed under the microscope and the small HHP Massage mattress was installed under the mouse skin, just in contact with this one (Figure 2).

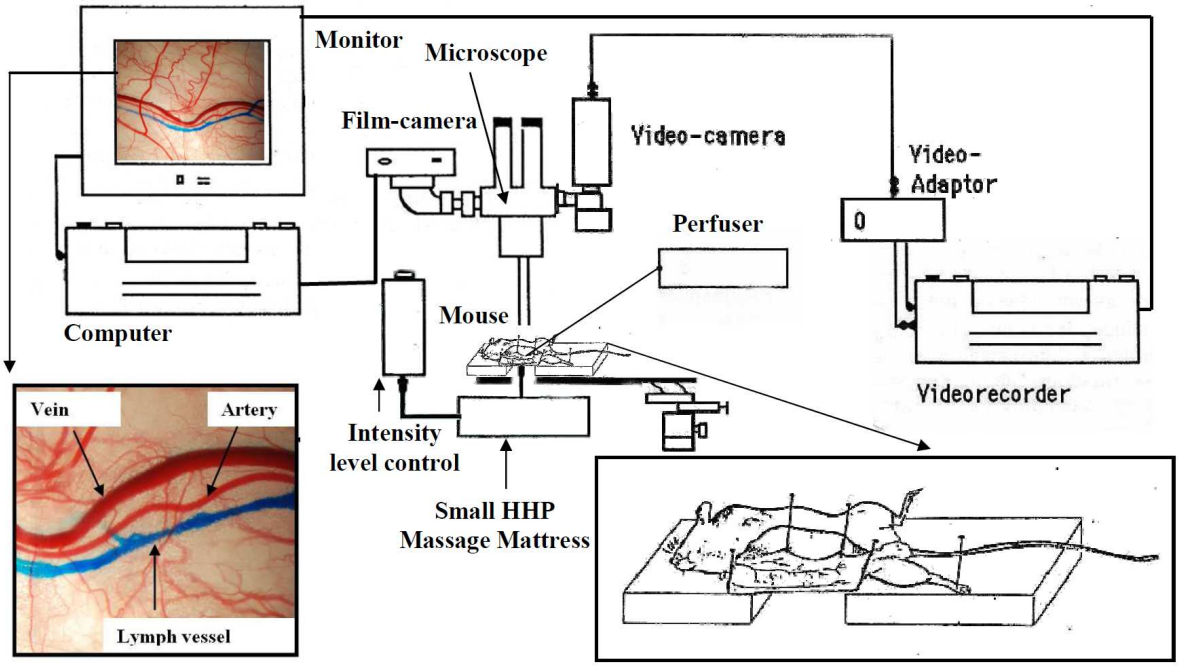


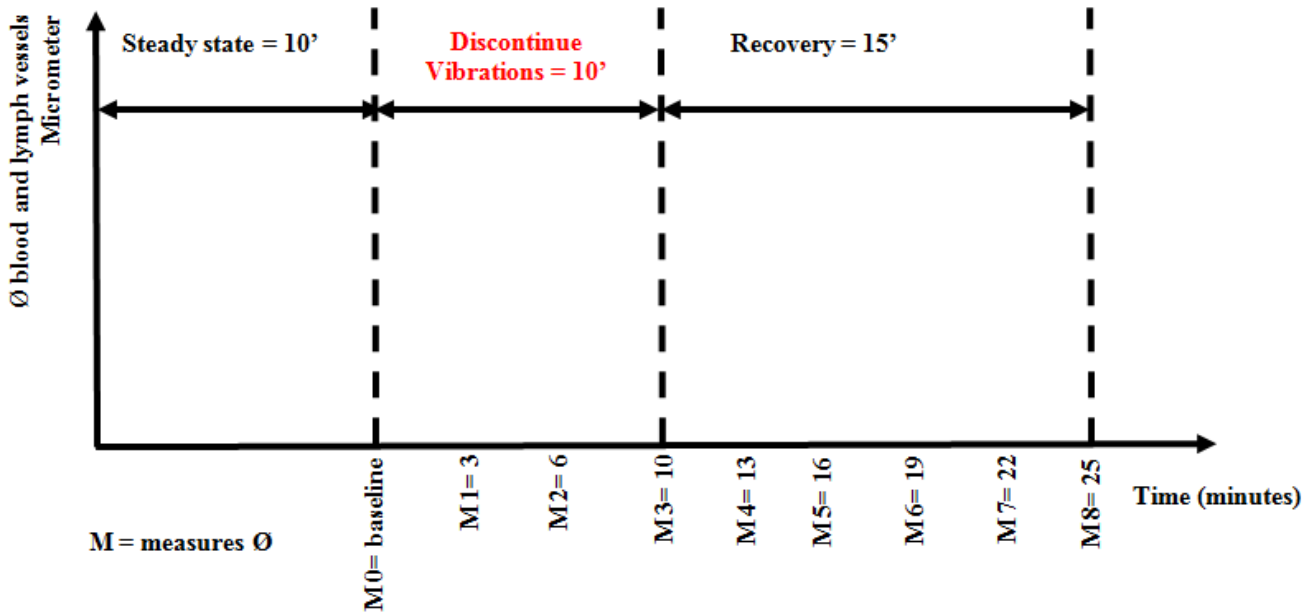
Figure: 2: Material and device used for visualization of the subcutaneous vessels of half-abdomen of the mouse after colouring, under the microscope by the technique of transillumination.

After the mouse installation, photography of the microcirculation was taken under microscope (enlargement x40 x2) with the Nikon D80.
A 10 minutes steady state period was respected. Photographies were taken during this period.

Then, local vibrations at 30 Hz (level 3) lasted for 10 minutes. At the end of 3th, 6th, 10th minutes, photographies were taken. In order to take the photographies, the vibrations were stopped during 10 seconds (discontinue vibrations).
Once vibrations ended, additional photographies were made for 15 minutes of recovery, at the end of 3th, 6th, 9th, 12th, 15th minutes.

During the experimentation, if it was necessary, the skin of the mouse was humidified with physiological solution (Na Cl 0.9%), to avoid skin dehydration, and a movie of the experiment was realized.

At the end of the experiment, the animals were euthanized.



Graph 1: This graph illustrates the experimental protocol which was used.

Results

The various diameters of the vessels are measured on the photographs taken at various times of the experimentation. The results are presented in the table 1.

Microscopic study: effects of the vibrations on blood and lymph vessels

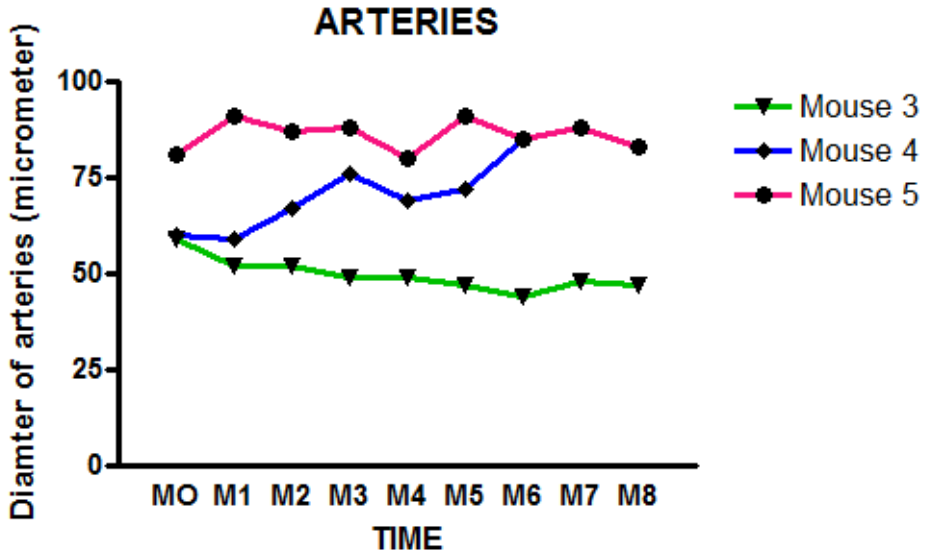
S = mouse — Level vibration: 3 (± 30Hz)

	Step	Time	S 1 ø microm			S 2 ø microm			S 3 ø microm			S 4 ø microm			S 5 ø microm			S 6 ø microm		
			A	V	L	A	V	L	A	V	L	A	V	L	A	V	L	A	V	L
Filmed experiments	Steady state	pdt 10'													5 min			5 min		
	Vibrations	0'= M1	/	176	114	/	193	179	59	253	158	60	191	116	81	184	87	/	137	54
		3'= M2	/	183	87	/	207	181	52	257	117	59	193	126	91	205	78	/	133	51
		6'=M3	/	184	70	/	214	187	52	263	81	67	201	112	87	213	99	/	133	63
		10'= M4	/	174	141	/	210	184	49	267	94	76	207	109	88	219	63	/	144	56
	Recovering	13'= M5	/	189	157	/	217	195	49	278	68	69	226	109	80	216	25	/	144	60
		16'= M6	/	181	166	/	221	189	47	270	78	72	217	126	91	223	36	/	151	60
		19'= M7	/	198	183	/	245	179	44	267	57	85	218	116	85	213	31	/	151	65
		22'=M8	/	176	156	/	238	182	48	281	71	/	/	/	88	212	23	/	151	68
		25=M9	/	199	189	/	229	198	47	263	60	/	/	/	83	178	20	/	164	60

Table 1: Values of diameters (micrometer) of blood and lymph vessels during the different steps of experiment

For each kind of vessel, we obtain the following graphs.

1/ Arteries



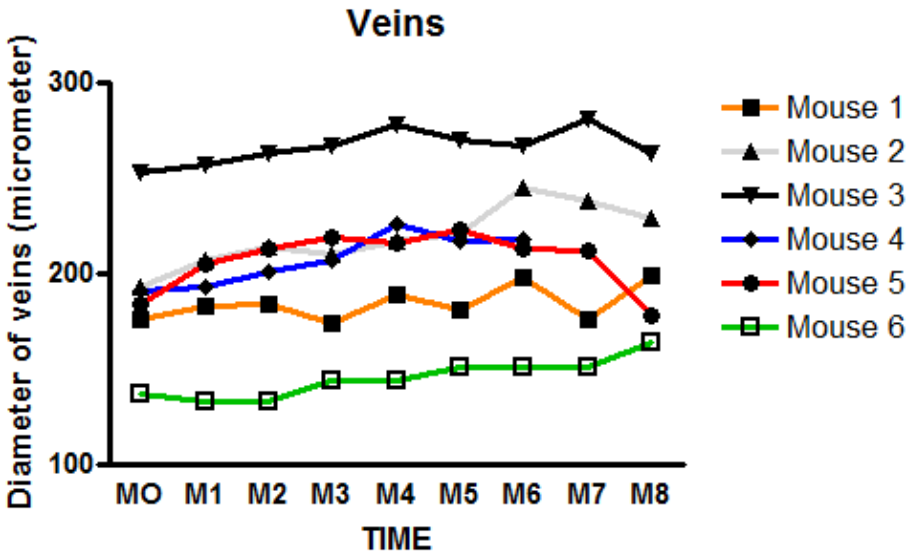
Graph 2: Values of the diameters of arteries during the experimentation

For technical reason, it is very difficult to observe in the same microscopic view, all vessels (artery, venous and lymph vessels). For mice 1, 2 and 6, we could not measure the diameter of the arteries.

On the graph 1, we can observe that the artery diameters are relatively stable (2 cases out of 3)

For the variable results of the mouse 4, explications are done in the discussion.

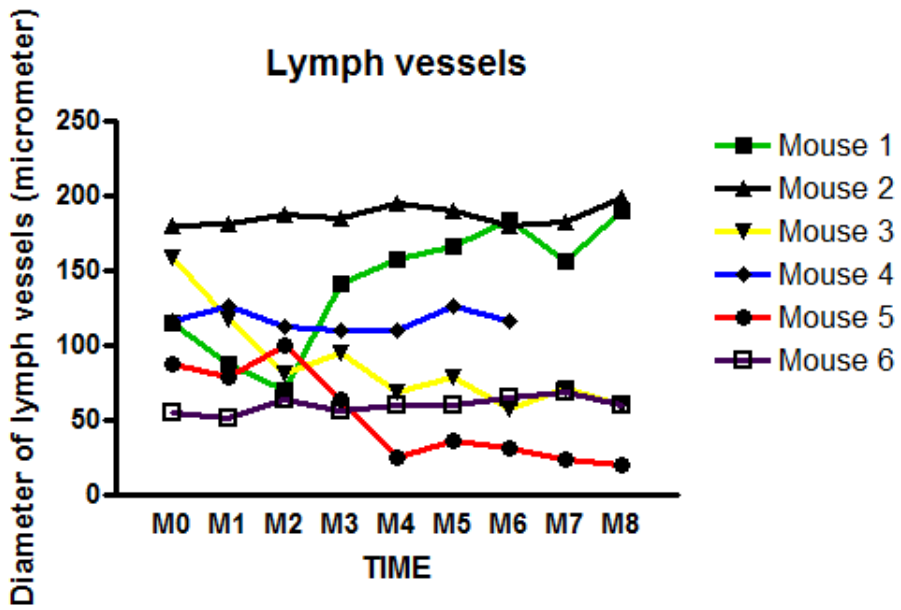
2/ Veins



Graph 3: Values of the diameters of veins during the experimentation

According to the curves on the graph, it seems there is a small increase in the diameter of the veins during the vibrations (M1 and M2), excepted for the mouse 6. During the recovery, the results are different for each mouse, and it is difficult to conclude.

3/ Lymph vessel



Graph 4: Values of the diameters of lymph vessels during the experimentation

We obtain various results according the mice:

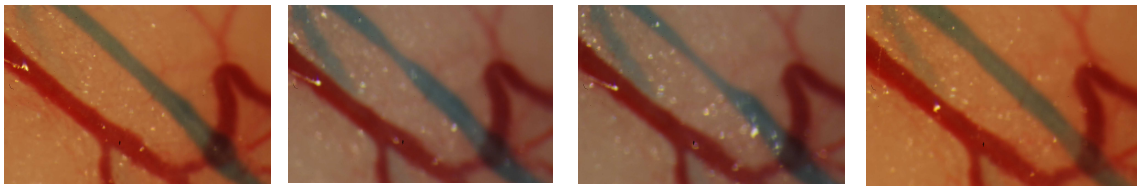
a/ For the mice 2, 4 and 6, the diameter of the lymph vessel is unchanged during the experimentation.

b/ The mice 3 and 5 are practically the same general type of the curve.
We can observe for these mice, a decrease of the vessel diameter during vibrations (excepted M2) and a chronic lymph vessel spasm during the recovery.

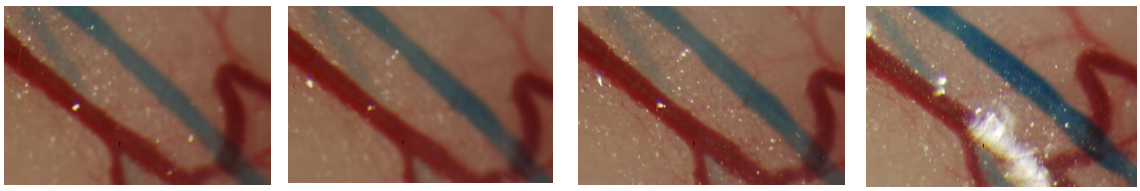
c/ The diameter of the mouse 1 vessel decrease during the vibrations and increase during the recovery.

Examples of microscopic views

Mouse 1

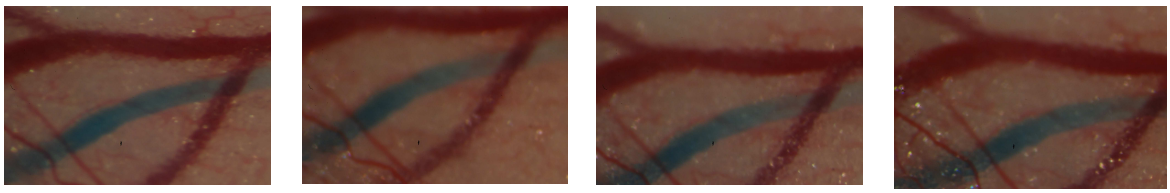


Before vibrations | after 3’ vibrations | after 6’ vibrations | after 10’ vibrations

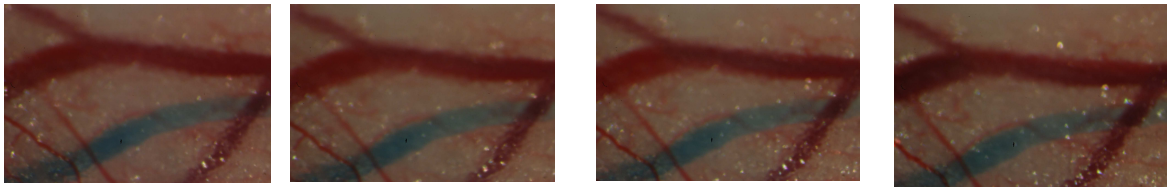


3’ recovery | 6’ recovery | 9’ recovery | 15’ recovery

Mouse 2

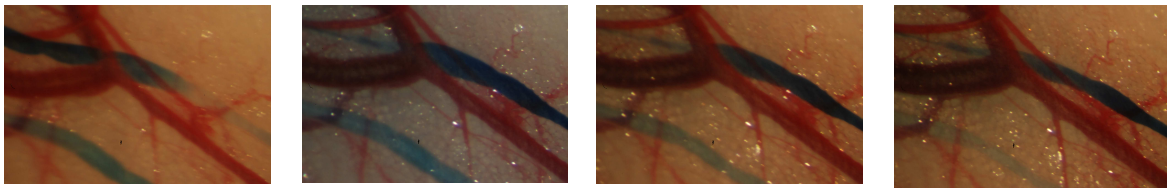


Before vibrations | after 3’ vibrations | after 6’ vibrations | after 10’ vibrations

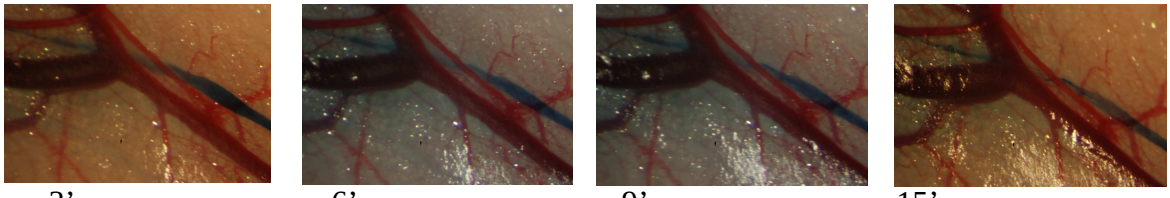


3’ recovery | 6’ recovery | 9’ recovery | 15’ recovery

Mouse 3



Before vibrations | after 3’ vibrations | after 6’ vibrations | after 10’ vibrations



3’ recovery | 6’ recovery | 9’ recovery | 15’ recovery

According to us, the measurement of the diameter of the vessel is not enough to understand the evolution of lymph vessels during the vibrations and the recovery.
The progression of the lymph in the lymph vessel is done with the contraction of lymphangions (contractility unit of the lymph vessels). The diameter of the lymph vessel varies during the time according to the lymphangion contraction.

It is the reason why, we filmed the experiments under the microscope
The results are presented with the table 2.

Vibrations level 3 (± 30Hz)

Steps	S1	S2	S3	S4	S5	S6
Steady state	?	0	7	0	0	0
Vibrations	16	not visible	not visible	not visible	2	?
Recovery	2	0	8 + x vasosp	0	3 + x vasosp	Mot + contrac?

Table 2: Number of visible contractions of the lymphangions during various steps

It is very difficult to observe normal lymphangion contraction under the microscope because you must find a 'very active lymphangion' and it is not always possible (3 cases out of 6).

It is very difficult to calculate correctly the number of contractions during vibrations (2 cases out 6), because the mouse skin vibrate also.

We cannot conclude about the presented results in the table 2, because the results are very different and the number of mice is small.
Sometimes, there is a vessel spasm after vibrations (mice 3 and 5), and it is very difficult to observe the real lymphangion contractions (example mouse 3 page 12).
Sometimes, there is only small movements and not real big contractions (mouse 6).

Discussion

Materials and methods

Discontinue vibrations:
We chose to deliver **discontinue vibrations** at the level 3 (30 Hz) to have the same experimental protocols with the anterior studies (Lohman in 2007 and Maloney-Hinds in 2008), and to take microscopic photographies at various times.

It is a good choice for the study of the diameter of blood vessels but no for the study of lymph vessels, because there are lymph vessels contraction, and an important variation of the lymph vessels diameters. It is the reason why, we filmed the experiments to obtain another parameter about lymph vessels (number of contraction).

Measurement method of vessel diameter:
The measurement method of vessel diameter is very precise. We can explain the variability of some results (about arteries) with the quality of the photography. In fact, if the quality of the photography is not very good, the precision of the diameter measurements will be less correct.

When you take a microscopic photography, it is difficult to obtain in the same picture the artery, the vein and the lymph vessel, because you must choose a microscopic view with a correct lymph vessel which seems to move.

For each mouse, we take always the measurement on the same place on the vessels

Stability of the experimental device:
We used two type of experimental device, because stability of the dissection board was not satisfactory.

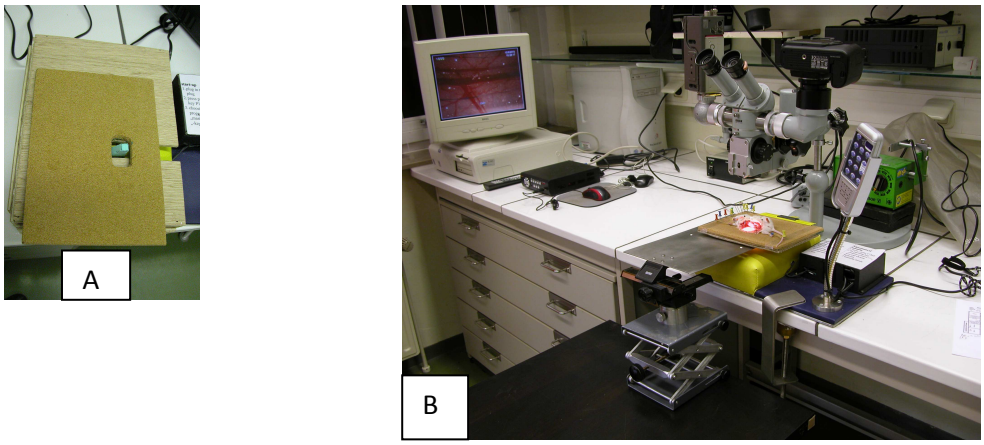


Fig 3: Primary (A) and last (B) type of stability system of the dissection board.

Finally, we used an elevator system to adjust the height of the dissection board (Fig 3), because the wood support (A) also vibrated during the vibration period.

Conclusion

For the study of the multidirectional vibrations on blood vessels, our experimental protocol with discontinue vibrations is correct, but not for the study on the lymph vessel.

For the lymphatic study, we propose to continue to record the experiments and to use various continue vibration times without vibration interruption.

References

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