IN VIRTO TESTS

90-R19

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LABORATORY OF EVALUATION OF IMPLANTABLE MATERIALS

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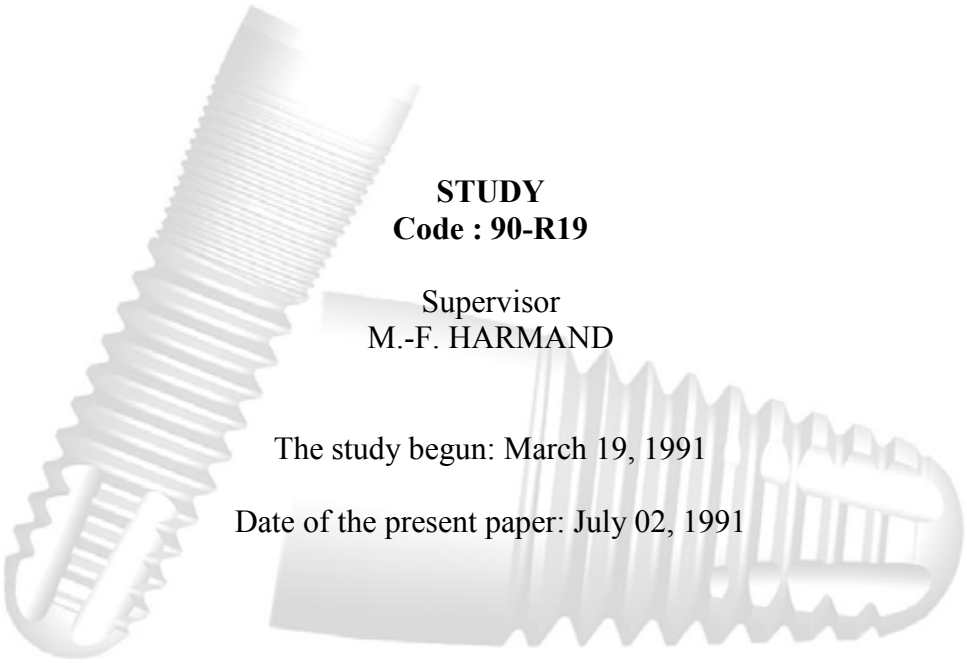

STUDY
Code : 90-R19

Supervisor
M.-F. HARMAND

The study begun: March 19, 1991

Date of the present paper: July 02, 1991

MANUFACTURER

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Study of the cytocompatibility of a PVDF

The study is done according to the norm NF S 90-702

- With an extracting liquid

- By direct contact

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Laboratory of evaluation of implantable materials

Montesquieu Technopole

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Ref. 91-R-C1 Martillac, July 02, 1991

Control Certificate of biocompatibility

Type of control: " "in vitro" Evaluation of the cytotoxicity of a PVDF"

Inquirer: SOFAMOR

Controlled product:

PVDF, code 080291-1

Protocol used: Norm NF S 90-702 on an extracting liquid obtained according to NF S 90-701 and by direct contact with fibroblasts of human gum.

Criteria of conformity: cell counting

- No significant difference in presence of the extracting liquid
- No diminution of the cell number by direct contact

Trying period: 19 March 1991- 02 April 1991

Conclusion: Conform material- No cytotoxicity

M-F HARMAND
Supervisor

1. Study of the biomaterial in presence of an extracting liquid

1.1. Preparation of extracting liquids

The extracting liquid, which corresponds to the PVDF, has been realized according to the NF S 90-701 norm.

- Extracting vehicle: culture medium (IMDM: Iscove's modified Dulbecco's medium)

- Extracting conditions: 120 hours at 37°C at 5 cm^2 of surface from the material/ml of the extracting vehicle.

We have also made a "blank» made of the extracting vehicle submitted to the extracting conditions in the absence of the material.

- Characteristics of extracting liquids:

  The pH of the extracting liquid: E has been measured, the pH of the "blank" and of the extracting vehicle too (EV).

	E	Blank	EV
PH	8.55	7.80	7.58

The extract E shows a basic pH when compared with the extracting vehicle. No change of volume has been observed.

1.1 Preparation of cultures

The cultures used are fibroblasts of human gum (FGH/4) at the 4^{th} passage. The absence of mycoplasms has been verified.
The cells are divided up in multiwells plates of 24 cupules (diameter 15.5 mm) at the rate of 70 000 cells/wells that is at subconfluence. The culture medium is the IMDM supplemented by 10% (v/v) of foetal calf serum (FCS).

Three days after the seeding, we make sure that the cells are at confluence and do not show any morphological signs of alteration.

1.2 Study of the cytotoxicity

The culture medium is sampled, the extracting liquid E and the "blank" are diluted in the culture medium at concentrations of 1,10,50 and 100% then supplemented by 10% of FCS.

Then it incubates for 72 hours at 37°C in moist air containing 5% of CO_2 at the rate of 4 wells by set.

At the end of the incubation period, the culture medium is sampled in each well and the study of the cell viability is done with the "Trypan Blue Test" (LEMI procedure n°13.6): 300 µl of a trypsine solution of (0.1 % (p/v) in Hank's without Ca++, nor Mg++ activated 30 minutes at 37°C) are put in contact with the cell layer until the total detachment of cells. Then we add 300 µl of Tryphan Blue solution (0.2% (p/v) in NaCl 0.15 M) and then it incubates for 2 minutes. Then under the microscope, we count the living cells (not coloured), in Malassez cells.

1.4 The results

The results are shown in the table 1 of figure 1.

No significant difference has been observed between the dilutions of the extract E and the corresponding dilutions of the "blank".

1.5. Interpretation

No cytotoxicity has been shown in the presence of the extracting liquid for the PVDF material (LEMI Code 080291-1) within the NF S norm 90-702.

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Extract – 72 hours

living cells (number of cells/cm^2)

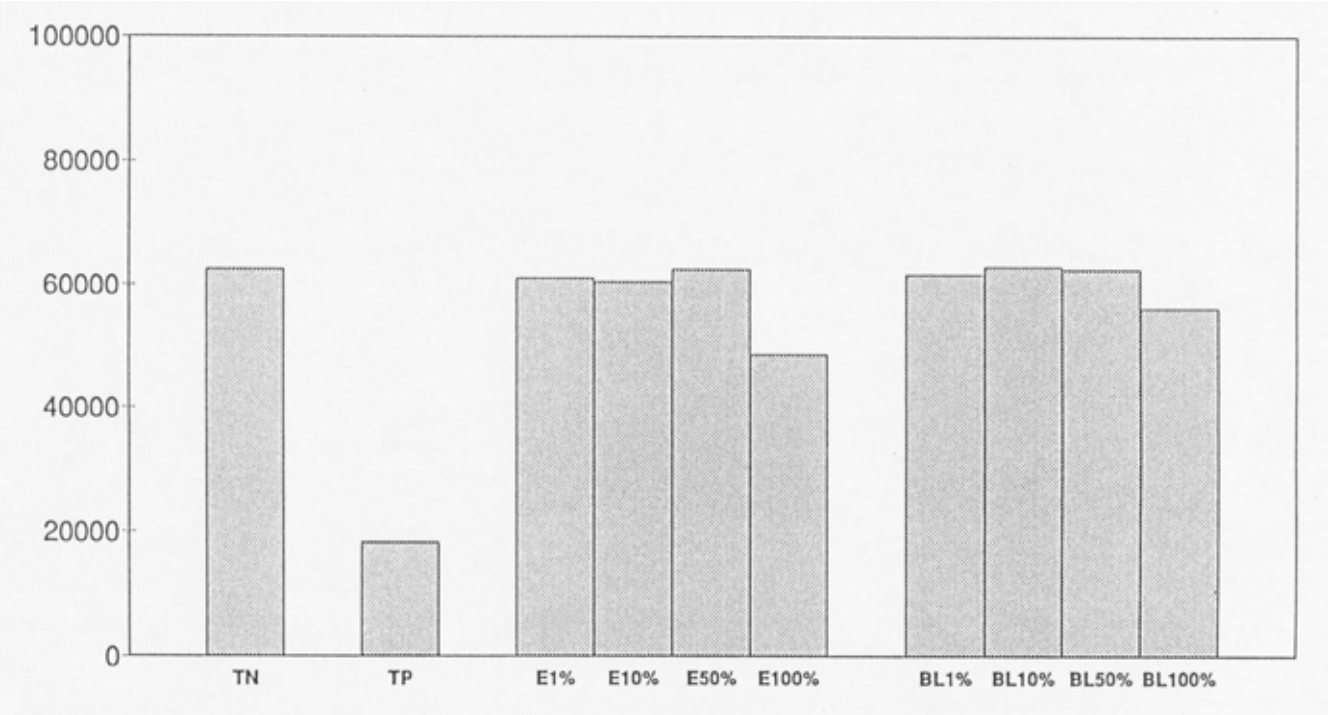
Percentage	0	1	10	50	100
Negative control	65 000 64 500 61 200 59 400 62 550 ± 2 345	_	-	-	-
Blank	-	57 900 53 100 63 000 73 000 61 750 ± 7380	55 800 74 100 64 800 57 300 63 000 ± 7260	70 200 66 300 64 800 48 900 62 550 ± 8 125	55 500 65 100 55 800 48 000 56 250 ± 5 865
E	-	58 800 61 200 67 500 57 000 61 125 ± 3970	59 400 64 500 61 800 56 400 60 525 ± 2 990	64 200 54 300 57 900 73 800 62 550 ± 7 400	55 800 53 100 47 400 38 400 48 675 ± 6 660

Table 1

Number of cells/cm^2

STUDY OF THE CYTOTOXICITY ACCORDING TO NF S 90-702 (EXTRACT)

Figure 1



2. Study by direct contact with the material

2.1 Preparation of cultures

The used cultures are fibroblasts of human gum (FGH/4) at the 4th passage. The absence of mycoplasms has been verified.

The cells are divided up in multipuits plates of 35 mm of diameter at the rate of 350 000 cells/wells (35 000 cells/cm^2) that is at subconfluence and they do not show any morphological signs of alteration.

2.2 Study of the cytocompatibility

- The material (LEMI code 080291-1) is delicately put in the center of the culture.
- The "negative control" cultures (without sample) are done simultaneously.
- At the end of the incubation period (24 hours) at 37°C in moist air containing 5% of CO_2, the counting of dead and living cells is done like for the previous study in presence of extracting liquids.

2.3. The results

The results are shown in table 2 and figure 2.

No significant difference has been neither shown, nor any morphological sign of cytotoxicity.

2.4. Interpretation

The PVDF material (LEMI Code 080291-1) studied in direct contact is not cytotoxic within the NF S norm 90-702.

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Direct Contact-24 hours

	living cells / cm²	Dead cells / cr
Negative control	39 600 33 200 34 400 35 735 ± 2 780	600 800 600 665 ±
PVDF	34 400 41 200 39 200 38 265 ± 2 855	400 1400 1000 935 ± 41

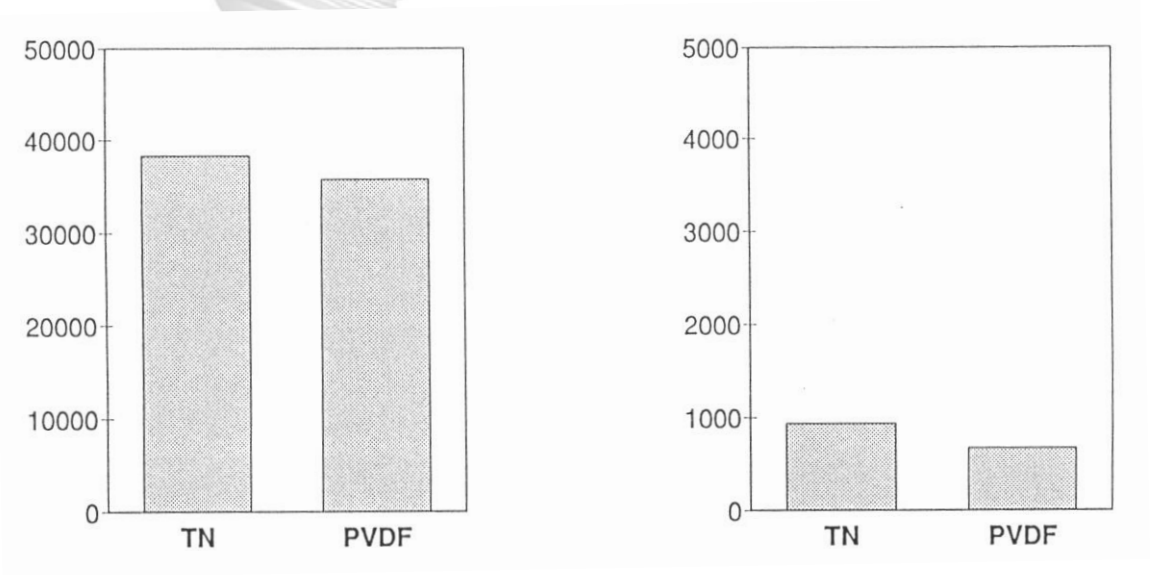
Table 2

Number of cells / cm2

LIVING CELLS
NC
PVDF
DEAD CELLS
NC
PVDF

STUDY OF THE CYTOTOXICITY ACCORDING TO NF S 90-702 (Direct Contact)

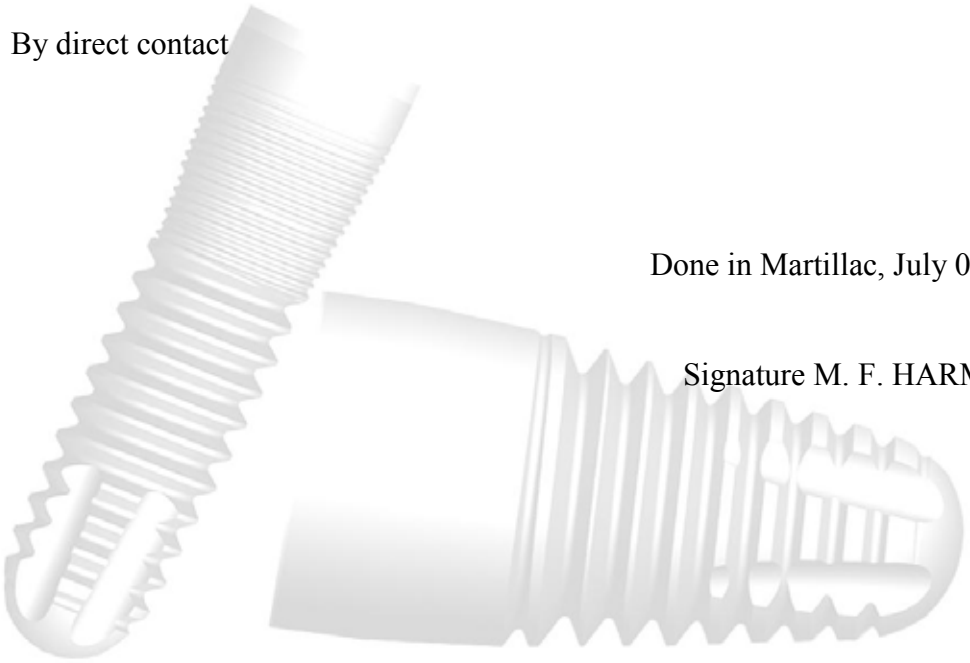
Figure 2



3. CONCLUSION

Within the NF S 90-702 norm “In vitro evaluation of the cytotoxicity of materials and medical measures” the PVDF material (catheter code 080291-1) shows no cytotoxicity for the fibroblasts of human gum:

- In presence of an extracting liquid
- By direct contact



Done in Martillac, July 02, 1991

Signature M. F. HARMAND