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Biocompatibility of Candidate Materials for the Realization of Medical Microdevices

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Abstract— **The propulsion of ferromagnetic micro-carriers in the blood vessels by magnetic gradients generated from a Magnetic Resonance Imaging (MRI) system is of special interest for targeted interventions such as chemotherapy or chemo-embolization. As such, Fe-Co alloys for its highest magnetization saturation, and single crystal Ni-Mn-Ga powder and Terfenol-D for their deformation in magnetic field are evaluated for their biocompatibility. The toxicity of these materials is evaluated with MTT cell viability tests. The tests show that Fe-Co (Permendur and Vacoflux 17) alloys are toxic within 24 hours while the single crystal Ni-Mn-Ga powder becomes toxic after 48 hours. The Terfenol-D, despite its high degradation, has 90% cell viability after 72 hours. These results indicate that such candidate materials to be considered in untethered micro-carriers or devices in the blood vessels, would require, depending upon the time spent in the blood vessels, further processes to be viable for such applications.**

Index Terms— **Biocompatibility, Fe-Co alloys, Terfenol-D, single crystal Ni-Mn-Ga, medical microdevices, MTT test**

I. Introduction

The aim of the Magnetic Resonance Submarine (MR-Sub) project is the automatic navigation of ferromagnetic entities in the blood vessels for targeting regions of the cardiovascular networks that are inaccessible or at high risks with existing modern interventional tools. As such, the concept relies on the propulsion or steering forces induced on a ferromagnetic core embedded in microdevices or carriers designed for a specific application. Gradient coils embedded in a clinical MRI system are used to induce such force on the ferromagnetic core. As the size of the ferromagnetic core decreases, more gradient strengths are required to induce sufficient force. Because of the limit in the maximum gradient amplitude that can be generated due to technological constraints, the saturation magnetization of the ferromagnetic core [1] becomes strategically important, especially for operations in the capillaries. As such, Fe-Co alloys such as Permendur and Vacoflux 17 with the highest magnetization saturations as depicted in Table 1 are of special interest in this particular context.

TABLE I
FERROMAGNETIC MATERIALS WITH THEIR SATURATION MAGNETIZATION

	Material	Saturation magnetization (T)
1	Fe-49%Co (Permendur)	2.40
2	Fe-17%Co (Vacoflux 17)	2.20
3	Iron	2.16
4	Cobalt	1.72
5	$Nd_2Fe_{14}B$	1.61
6	Fe-47,5%Ni	1.5
7	AISI 304L stainless steel	1.28
8	Fe-80%Ni	1.04
9	Fe-50%Ni-10%Cr	0.75
10	Cu_2MnAl	0.70
11	Nickel	0.61
12	Fe_3O_4	0.60
13	$CoFe_2O_4$	0.50
14	$BaFe_{12}O_{19}$	0.48
15	$NiFe_2O_4$	0.34

Another interesting aspect for the implementation of some devices is the capability of deformations when placed in a magnetic field, a phenomenon also known as magnetostriction. As such, two materials are considered: single crystal Ni-Mn-Ga alloys [2] and $Tb_{0.27}Dy_{0.73}Fe_{1.95}$ giant magnetostrictive material, also known as Terfenol-D ($Tb_xDy_{1-x}Fe_y$). This study is a continuation of the (3-(4,5-dimethylthiazote-2yl)-2,5-diphenyl tetrazodium bromide) (MTT) results on polycrystalline Ni-Mn-Ga which shows the toxicity of this alloy. The toxicity of Ni-Mn-Ga alloy is evaluated here for different shapes and crystalline structures.

II. Materials & Methods

Vacoflux 17 and Permendur are Fe-Co alloy with 81%Fe, 17%Co, 2%Cr and 49%Fe, 49%Co, respectively. The samples were sized by Electrical Discharge Machining (EDM) prior to the MTT tests. The weight of each sample

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was 2 grams. Every sample was cleaned with acetone followed by methanol in an ultrasonic bath during 10 minutes.

Single crystal Ni-Mn-Ga powder has the following composition: 48.6 at% Ni, 31.5at% Mn, 19.9at% Ga.

For the $Tb_{0.27}Dy_{0.73}Fe_{1.95}$, samples in powder form and with different bulk sizes (sample 1: length 23mm and sample 2: length 10mm) and a thickness of 2.08mm were used during MTT tests.

Ethylene Oxide was used for the sterilization of the Ni-Mn-Ga and Terfenol-D samples. Five days after sterilization, the ethylene oxide residues were removed from the samples. Half of the Fe-Co samples were sterilized with ethylene oxide while the remaining samples were sterilized by ultra-violet rays. For UV sterilization, the samples were put in a sterile host during 24 hours [3]. Prior to UV sterilization, the samples were cleaned with acetone and isopropanol under agitation.

Dulbecco's Modified Eagle's Medium (DMEM) (Sigma Chemical Co.) was added on the samples and placed in an incubator (37°C, 95-5% O_2-CO_2 humidified atmosphere) during five days. To avoid degradation of the powder, the samples were not shacked during extraction. After extraction, the medium was centrifuged (1200rpm, temperature= 4°C) during 5 minutes. For extraction, 0.1 g/ml of medium for the powder and 0.2g/ml for the bulk samples were used.

Mouse fibroblast cells L929 were used. For the Ni-Mn-Ga and Terfenol-D samples, cells of different years (2003 and 2004) were used to avoid the fact that the results of toxicity can be induced by the age of the cells. The cells were cultured at 37°C, 95-5% O_2-CO_2 humidified atmosphere in DMEM, supplemented with 3.7g/l of sodium bicarbonate, 10% heat-inactivated (56°C for 30 min.) Fetal Bovine Serum (FBS, Gibco laboratories) and 1% penicillin-streptomycin solution (Gibco laboratories) [4].

The MTT test is based of the capacity of the living fibroblasts to synthesise (3-(4,5-dimethylthiazote-2yl)-2,5-diphenyl tetrazodium bromide) into formasan crystals. by the mitochondria [5]. The crystals are released from the cells with acidified isopropanol. The optical density of each well is measured. The cells are seeded in 96 well culture plates at a density of 5×10^4 cells/200µl cell culture liquid. After 24 hours, the medium is removed from the wells and replaced by the extracts. The test is done for three time periods: 24, 48 and 72 hours. As negative control, only cells with the medium in the wells are used. The optical density is determined by a microplate reader operating at 570 nm.

III. Results

Fe-Co alloys:

The cell viability is higher for the Vacoflux 17 than for the Permendur (Fig. 1). Despite this difference, the cell viability is not higher than 72% after 24hr. For these materials, it should be noted that the viability is decreasing significantly after 24hr of incubation with cells. This result is confirmed by the morphology of the cells (fig 5). The two sterilizations were efficient and there is no significant difference between the two processes.

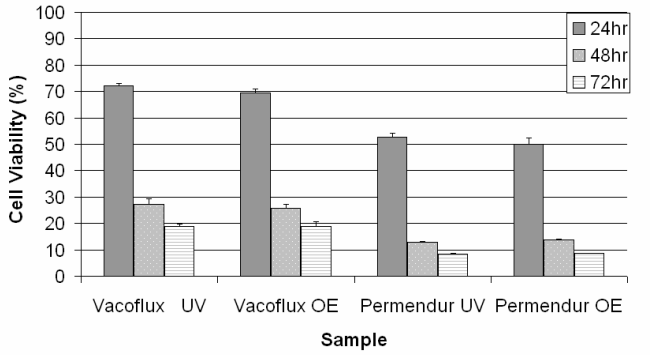


Fig 1. L-929 viability after exposure to Fe-Co extracts. Vacoflux UV means Vacoflux 17 sterilized by UV and Vocoflux OE means vacoflux 17 sample sterilized with Ethylene Oxide.

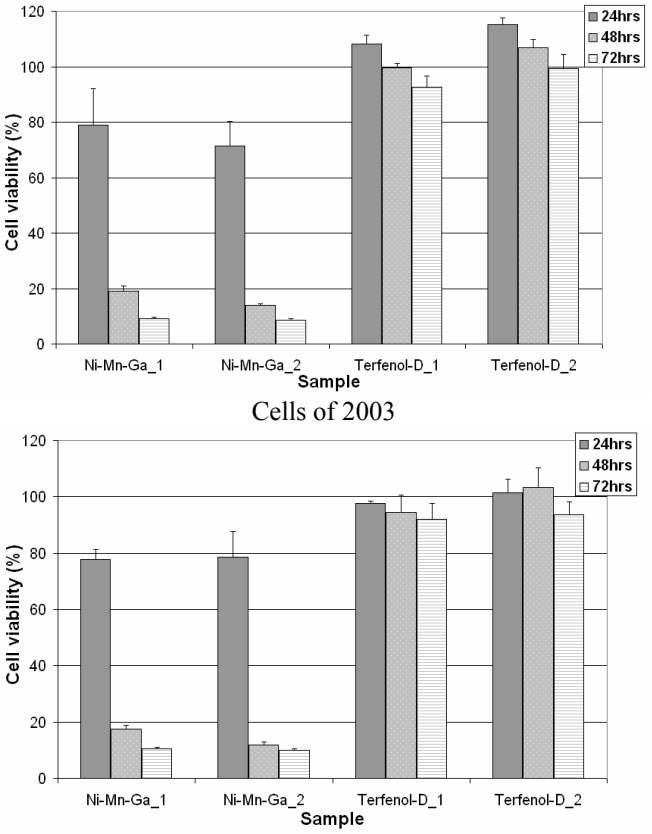


Fig 2. L-929 (2003) viability after exposure to single crystal Ni-Mn-Ga powder (Ni-Mn-Ga_1 & Ni-Mn-Ga_2) and $Tb_{0.27}Dy_{0.73}Fe_{1.95}$ samples (Terfenol-D_1 = sample 1 & Terfenol-D_2 = sample 2).

(a)

(b)

Fig 3. $Tb_{0.27}Dy_{0.73}Fe_{1.95}$ samples after the extraction during five days: (a) sample 1 and (b) sample 2.

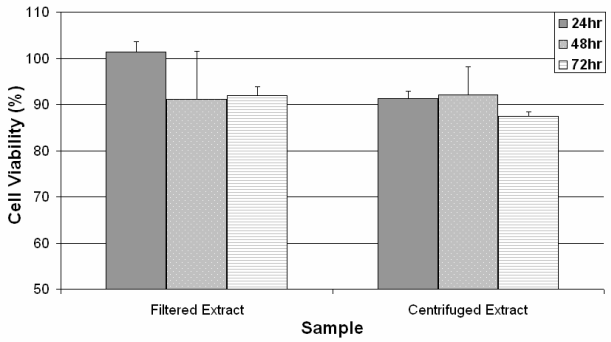
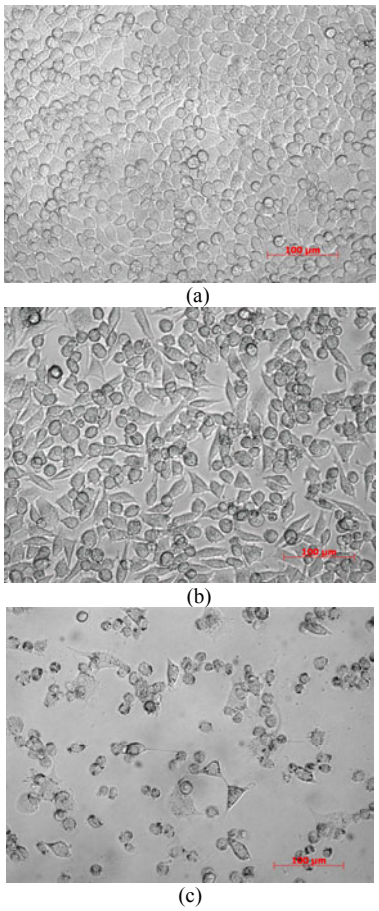


Fig 4. L-929 viability after exposure to $Tb_{0.27}Dy_{0.73}Fe_{1.95}$ powder.



(a)

(b)

(c)

Fig 5. L-929 pictures in the well of the 96 wells plate at 48hr of incubation. (a) L-929 as control, (b) L-929 cells with filtered extract of $Tb_{0.27}Dy_{0.73}Fe_{1.95}$ powder, (c) L- 929 Cells with Vacoflux 17 OE.

Single crystal Ni-Mn-Ga alloy:

There is no significant difference between the results obtained with cells from 2003 and the cells from 2004 (Fig 2). The optic density is the same between the two samples. The cell viability at 80% is quite acceptable at 24 hours (Fig 2). After 24 hours, the viability decreases very rapidly. This result shows that Ni-Mn-Ga is toxic for long period application in the body.

$Tb_{0.27}Dy_{0.73}Fe_{1.95}$ giant magnetostrictive material:

The cell viability for the Terfenol-D is very high. We note that the cell viability is decreasing sensibly with longer incubation time but remains acceptable. Despite the slight degradation of the samples (Fig 3), it appears not to be toxic. Figure 5 shows clearly the good viability of the cells by their morphology. These results are completed by further tests on Terfenol-D powder having the same composition as the bulk samples. With the powder, the effect of the centrifugation during the extraction was investigated. No significant difference was noticed for incubation time reaching 48 and 72 hrs. The cell viability remains very good.

IV. Discussion

This first test shows the cytotoxicity of the Fe-Co alloys on a long incubation time with cells. From this test, we cannot conclude on the origin of the cell viability difference between the two alloys. These results can potentially be explained by the percentage of Co in the samples. The cobalt is known to be toxic when it is released in the body [6,7]. For the Vacoflux 17, the presence of chrome gives to the alloy better corrosion behaviour, but this is still to be verified. Hence, the ions release is less than from the Permendur samples. These hypotheses can be validated by a corrosion test and an analysis of the corrosion solution by Inductive Coupled Plasma-Mass Spectroscopy (ICP-MS). Despite a lower saturation magnetization than the Permendur (2.2T vs. 2.45T), it seems that the Vacoflux 17 is the best candidate for the implementation of ferromagnetic devices.

It's clear that Ni-Mn-Ga cannot be used in the body in powder form except for period not exceeding 24 hours. The ions release from this alloy has to be evaluated because it can have an influence on the cell viability [8]. The results with Terfenol-D are very surprising after observing the state of the samples after the extraction process (Fig 3). The degradation products of this material seem to be not toxic. The limit of this test is that the composition of the products degradation is unknown. A test done with powder, known to increase the specific surface, has very good cell viability. The fact that there is no evidence of the toxicity on the bulk and powder material opens the possibility to use this material in the fabrication of medical microdevices. The main problem of this material is its poor mechanical properties beside applications of this material as a composite [9].

V. Conclusion

From its magnetic property, Vacoflux 17 is a good material to be embedded in microdevices being propelled in blood vessels by magnetic gradients generated by a clinical MRI system. Unfortunately, the cell viability is not high enough to use this alloy in the human body and require additional processes such as coating with titanium to increase the corrosion resistance and to prevent the release of toxic ions [10,11]. Haemocompatibility tests will be done to evaluate the blood reactions.

Ni-Mn-Ga alloy is a very interesting material for the development of new microdevices operating in a magnetic field. Because of high toxicity after 24 hours, this material cannot be used without further processing. A very promising alternative is to develop a composite-based Ni-Mn-Ga powder [12].

$Tb_{0.27}Dy_{0.73}Fe_{1.95}$ giant magnetostrictive material offers good cell viability but its biocompatibility has to be proved by inflammatory tests. The degradation of this material indicates that it will be better used in a composite. Further tests of the magnetic properties at 37°C need to be evaluated.

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