**Cytotoxicity evaluation of magnetic fluid based on cell proliferations**

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**ABSTRACT Introduction** A magnetic fluid (MF) is widely used in a clinical field, and we have also developed MF seal systems for the blood pump as a motor shaft seal. For the clinical application, non-toxicity of the MF is needed, and we have reported the cell compatibility of some MFs by the direct addition of MF onto the cell culture [1, 2]. Those results showed that there were some negative cell proliferations, but they were not the critical level of cytotoxicity. During the improvement of MF seal systems, we have developed several types of MF specialized for our MF seal. The purpose of this study is to evaluate the cytotoxicity of MFs by using extracting solutions of MFs by the proliferation and the released LDH detection.

**Materials and methods** Five kinds of fluids, “exp. 16064”, “exp. 16065”, “exp. 16066”, “exp. 15067”(Ferrotec, Japan), and “LS-40”(Taiho Indust. Co., Ltd., Japan), were used for preparing the extracting solutions. Among the fluids, exp. 16066 was the solvent of exp. 16064, and exp. 16065. Five fluids were sterilized by autoclaving at 120 degC, for 25 mins. After the cooling process, each of five fluids was mixed individually with the low glucose Dulbecco’s modified Eagle medium (DMEM; Thermo Fisher scientific, US) at a rate of 1.2 mL of fluid per 24mL of medium in the cortical glass tube. Mixed medium groups were incubated for 1 week at 37 degC and 5% CO2 in moisture. After the incubation, those were centrifuged at 1500g for 15 min, and the separated volume media were moved to the newly prepared cortical tube.

Fibroblast-like cell, L929, was selected for the cytotoxicity evaluation that is referred to the ISO 10993-1 “Biological evaluation of medical devices”. Cells were pre-cultured with the DMEM with 10% calf serum and 1% antibiotics-antimycotics, and adjusted cell suspension was seeded into 2.0 x 104 cells/well at 48-well plate. Plates were pre-incubated at 37 degC and 5% CO2 for 1 day, and then after changing with the flesh medium, 200 L of extracting solutions were added to each 5 group of wells (n=6). Simultaneously, the negative control group without any extracting solutions and the positive control group with 10% triton-X were prepared for each plate. Multiple plates were prepared and cultured for 1 day, 2 days, 4days, and 6 days. After the incubation period, each plate was used to investigate the MTS assay(Cell Counting Kit-8, Dojindo, Japan) for the cell proliferation study depending on the metabolic activity of living cells and the LDH cytotoxicity assay(Takara Bio Inc., Japan).

**Results** Figure 1 shows the results of the cell proliferation ratio against the negative control. There were some reductions of proliferation from day1 to day2 in exp. 15067, and from day1 to day4 in LS-40. These cytotoxic results coincided with the results of LDH cytotoxicity (Table.1). The level of LDH cytotoxicity showed higher in exp. 15067 and LS-40 which continued after that of incubation periods.

**Discussion and conclusion** Among 5 kinds of MFs, 2 groups of extracts showed the decrease of cell proliferation and the increase of LDH cytotoxicity on early days of incubation. But at day6, the cell activation of those 2 groups was similar with others and the cell culture showed confluence. It was clear that the extracting solution of exp. 15067 and LS-40 showed the acute cytotoxicity. Those results indicated that the contents of MF were the key to insure the biocompatibility of MF.

***Keywords:*** *Magnetic fluids, biomedical use, cytotoxicity, cell proliferation*

**REFERENCES**

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Table 1 Results of LDH cytotoxicity (%)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **exp.16064** | **exp.16065** | **exp.16066** | **exp.15067** | **LS-40** |
| day1 | (under the detective value on assay) | | | | |
| day2 | 13.5 | 17.6 | 18.6 | 22.3 | 39.7 |
| day4 | 29.6 | 24.9 | 15.8 | 21.9 | 25.5 |
| day6 | 35.5 | 34.6 | 35.9 | 38.7 | 47.3 |



Fig.1 Results of cell proliferation studies