Magnetic Targeting of Human Aortic Smooth Muscle Cells for Rapid Closure and Healing of Intracranial Aneurysm/Pseudoaneurysm

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ABSTRACT

The aneurysm/pseudoaneurysm treatment requires a complete and definitive occlusion of the deffect from the parental artery to prevent bleeding. Current approaches such as coil embolization are still suboptimal for aneurysm sac occlusion, exhibiting various documented postsurgery recurrences. Asymetric bioactive stents with a magnetic surface able to attract magnetized cells may promote a rapid aneurysm treatment by bringing the aneurysm neck. An efficient cell internalization of nanoparticles is a perrequisite to address the feasiability of these approaches. In this study, cell magnetization using low pulse and low voltage electroporation parameters are assessed, and their effects on cell viability, genotoxicity, and nanoparticle internalization are shown.

Keywords: magnetic targeting, electroporation, iron oxide nanoparticles, vascular healing, aneurysm treatment.

1 INTRODUCTION

Endovascular treatment of intracranial aneurysms through the use of platinum coils has shown to yield excellent clinical results to prevent aneurysm rebleeding. However, its use is still controversial because of the difficulty to get occlusion of large aneurysms[1]. Several mechanisms have been proposed to address the current disadvantages of detachable coils without achieving an adequate treatment for their complete occlusion, and to prevent arterial stenosis after device placement [2, 3, 4].

A novel approach is proposed in this study using a magnetic strategy on the covered stent design. It consists of a patch facing the aneurysm neck defect capable of reducing the flow inside it and bridging the aneurysm neck. The patch is thought to have the ability to attract locally magnetized smooth muscle cells and promote the wound healing through rapid cell migration, adhesion, proliferation and deposition of the extracellular matrix, which are essential requisites for vessel integrity and vascular repair [5].

To test the feasibility of this approach, we have enhanced the cellular uptake of nanoparticles via electroporation for magnetic cell delivery. In that sense, improved cell guidance and retention on the patch will be conferred for rapid favorable healing of the aneurysm neck. The electroporation technique is a method of cell membrane permeabilization that uses short and intense electric pulses to deliver foreign molecules into cells. This technique is widely used in clinical practice to improve drug delivery for cancer treatment, as well as in biotechnology to introduce genes into living cells[6, 7].

2 MATERIALS AND METHODS

2.1 Cells

Human aortic smooth muscle cells (HASMCs, cat. # C-007-5C, Gibco® Life Technologies, Carlsbad, CA) were harvested with approximately 90% confluence and the cellular density and viability were determined using Countess® Automated Cell Counter (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. 9.7x105 live cells/mL were counted with a viability of 95%.

2.2 Electroporation of HASMCs

Aliquots of 500 µL of cell suspension at a density of $6x10^5$ live cells/mL were placed into electroporation cuvettes (640-45-0126, BTX Harvard Apparatus, Holliston, MA), and pulsed in Phosphate Buffered Saline (PBS) without Calcium or Magnesium (cat. # 17-516F, Lonza, Walkersville, MD). 2 µL of iron oxide nanoparticles in water of 30 nm size were added to each cell suspension, except for the negative control (SHP-30-0010, Ocean Nanotech LLC, Springdale, Arkansas). The electroporation process was carried out utilizing a BTX 830 square wave electroporator (Genetronics, San Diego, CA), with low voltage and low pulse parameters (see Table 1). After electroporation, HASMCs were undisturbed during 10 min, and subsequently seeded under normal culturing conditions (37.0°C, 95% air, 5% CO2).

Sample	Field Strength (V/cm)	Pulse Length (μs)	No. of pulses
S1– Untreated cells	---	---	---
S2	500	100	4
S3	400	100	4
S4	200	100	4
S5	200	200	4

Table 1: Spectrum of conditions tested for SPION loading of HASMCs using electroporation technique. Cells untreated correspond to HASMCs not subject of magnetic loading with SPIONs and/or electroporation.

2.3 Cell Viability

The viability of the cells was determined at 24h, 48h and 5 days respectively by Trypan blue exclusion assay (cat. # C10314, Life Technologies, Carlsbad, CA), using a Countess® Automated Cell Counter (Life Technologies, Carlsbad, CA)).

Visual inspection of the HASMCs was conducted during 5 days under bright field illumination utilizing a Nikon inverted Diaphot fluorescent microscope with 10X and 20X objectives (Nikon Instruments, Melville, NY). In vitro cell images were taken of each sample well using a Nikon Coolpix 990 digital camera mounted onto the Nikon inverted Diaphot microscope.

2.4 Genotoxicity

At day 5, Single Cell Electrophoresis or the CometAssay® was performed according to manufacturer's recommendations (cat. 4250-050-K, Trevigen, Inc., Gaithersburg, MD). Cells treated with 30% hydrogen peroxide in water were used as positive control in the assay, since they yield DNA migration. The DNA damage was quantified using CometScore™ software[8].

2.5 Prussian Blue staining

Cells were pulsed in presence of 16 μl of SPIONs in water (SHP-30-0010, Ocean Nanotech LLC, Springdale, Arkansas) at 500V/cm, 100μs and 4 pulses electroporation parameters, were incubated under normal culturing conditions during 24h. Cells treated under same conditions, except by electroporation, were underwent to passive uptake. HAMCs growing under normal culturing conditions were used as negative control in the test. At 24h, all the specimens were washed three times using DPBS. Afterward, they were fixed in 0.05% glutaraldehyde during 2 hours, and Prussian Blue staining was performed according with the manufacturer's recommendations (Accustain® Iron Stain, cat. # HT20-1KT, Sigma-Aldrich, St. Louis, MO).

3 RESULTS

3.1 Effect of Electroporation on Cell Viability

The influence of varying electroporation conditions on cell viability was performed by Trypan Blue exclusion assay at 24h, 48h, and 120h (see Table 2).

Sample	Viability (%)		
	24h	48h	120h
S1	76.33±12.08	88.50±5.01	80.67±7.04
S2	63.67±11.09	83.50±8.55	78.33±6.35
S3	79.00±16.90	77.83±7.68	82.67±3.78
S4	85.50±12.79	72.00±11.42	87.50±7.66
S5	79.00±18.74	82.00±4.82	82.00±5.55

Table 2: Percentage of Cell Viability calculated by Trypan Blue exclusion assay at 24h, 48h and 120h in pulsing HASMCs in presence of SPIONs.

The higher field strength setting (500 V/cm) showed to have the greatest impact on cell viability at 24h; however the percentage of cell viability remained above 75% at 48h and 120h. Applying 400 and 200 V/cm in conjunction with very short pulses (100 μs) exhibited the cell viability values steadier than higher field strengths (500 V/cm).

3.2 Effect of Iron Uptake on DNA Damage of HASMCs

HASMCs pulsed with commercial spions were investigated for potential DNA damage after 120h of incubation. Single-cell gel electrophoresis or the Comet Assay was performed, and a population of untreated and treated hydrogen peroxide cells was used as controls in the assay. The test is positive for increasing DNA migration from the cell nucleoid to the tail after electrophoresis, and indicates the presence of DNA strand breaks produced for detrimental effects of foreign molecules inside the cell.

At a density of 2 μg/mL of commercial SPIONs, no statistically significant differences were found between the untreated cell population and the tested samples either for percentage of DNA in tail (see Figure 1). This figure shows that % of DNA in tail for pulsed cells with SPIONs are majority ranging between 0-5%, whereas that the positive control possess a wide range above 10% of DNA in Tail that reaches up to 25%.

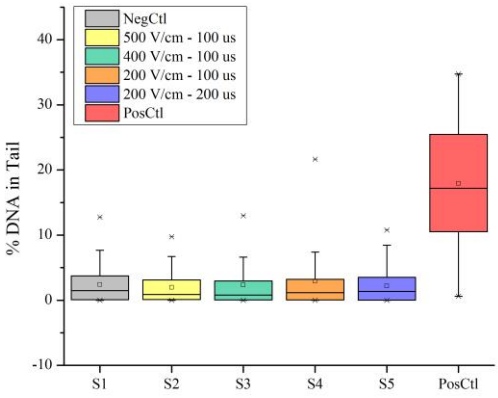
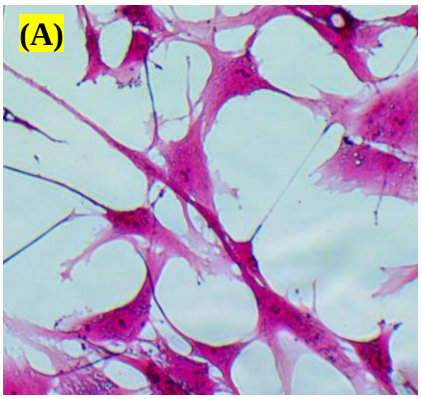


Figure 1: Graphs showing the % DNA Damage of pulsed HASMCs in presence of SPIONs after performing Comet Assay.

3.3 Iron Oxide detection inside the Cell

The internalization and distribution of SPIONs inside HASMCs were visualizaded via Prussian Blue Reaction, at 24h after electroporation, and passive uptake using SPIONs (see Fig. 2). Panel A shows untreated HASMCs and no presence of blue vesicles are evident insede the cell. Panel B corresponds to HASMCs subject to passive uptake, where SPION's buildups are display as giant dark vesicles. Panel C shows HASMCs after electroporation. It shows small blue vesicles distribuited around of the cytoplasm, outside of nucleous.



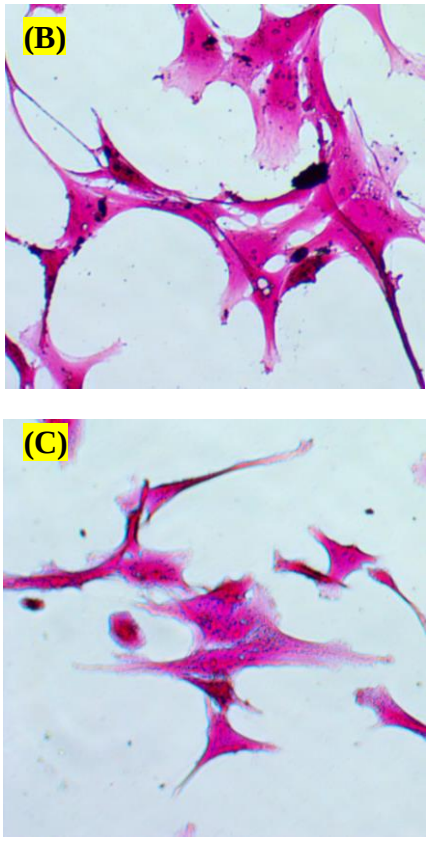


Figure 2: Prussian Blue Staining of HASMCs underwent to electroporation in presence of SPIONs and passive uptake under the same conditions and same concentrations of nanoparticles. The iron content is visualized forming blue and dark vesicles after Prussian Blue reaction. Panel A: untreated cells, Panel B: HASMCs undergone to passive uptake, Panel C: HASMCs after electroporation in presence of SPIONs.

4 DISCUSSION

In order to become HASMCs magnetic, SPIONs were delivered inside of their cytoplasm by electroporation. The low intensity and low duration pulse parameters were applied during the electroporation process in order to avoid cell lyses, but enough high to allow membrane permabilization, and consequently magnetic loading.

Magnetic labeling of HASMCs using commercial SPIONs by electroporation showed a high viability during 24h, 48h and 120h of tracking, reaching values of up to 87.5% at 120h for low field strength and low pulse duration parameters (200 V/cm, 100µs). However, sharp changes in cell viability were also registered in the timeframe of the test for almost all the electroporation parameters evaluated, passing from high to very low viability without keeping a clear trend. This could be explained by the poor sensitivity of the Trypan Blue exclusion assay to estimate cell viability. The same trend was observed for the untreated

control population, varying from very low viability at 24h, reaching higher values at 48h, and descending again to low viability values at 120h.

Despite absence of clear tendency, the cell viability for pulsed HASMCs was comparable to the viability reached for untreated cells, and no morphological changes were evidenced in culture during the days of the test (data not shown). These may indicated that HASMCs under the tested electroporation parameters were able to recover their cell membrane integrity and functionality.

The SPION internalization was limited to the cell cytoplasm instead the nucleous, in agreement with those observed in the Comet Assay resutls, where no genotoxicity effects were detected. Similar results were found by Walczak et al[9,10] using mesenchymal stem cells and neural stem cells, where no observable detrimental effects on the ability of the cells to differentiate were noticed after electroporation treatment in presence of SPIONs.

5 CONCLUSIONS

In this work has been showed an alternative methodology to render magnetic HASMCs without disrupt cell integrity and functionality. This has major implications for magnetic protocols, specially those based on cell targeting with applications in vascularization. Low voltage and low pulse electroporation parameters showed to have low negative effects on cell viability, and the results obtained were very close to those reached for the untreated cells. However, a more sensitivity test is needed to confirm these findings.

No genotoxicity effects on HASMCs were detected under the SPIONs concentrations assessed in the present work performing the Comet Assay at 120h of incubation. All the tested samples were characterized by compact nucleoids, indicating the no presence of DNA strand breaks on the pulsed and magnetic cell population. Besides, the percentage of DNA in Tail values for the tested samples were closer to those of negative controls rather than cell treated with hydrogen peroxide before performing Comet Assay.

Prussian blue staining showed eficient endosomal distribution of the SPIONs, without their presence in the nucleous.

6 ACKNOWLEDGMENTS

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