Pilot Study Assessing the Impact of Platelet Activation Electric Stimulation Protocols on Hematopoietic and Mesenchymal Stem Cell Proliferation

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***Abstract*— Recent research has shown that pulsed electric fields can successfully activate platelets ex-vivo; activation means here the release of growth factors and clotting. Typically, platelets are in a complex biological matrix, such as platelet rich plasma (PRP), which contains a variety of cell types. While specific electric pulses can activate the platelets, the impact of electric simulation on other cell types is an open question. The pilot study presented here focuses on evaluating electric pulse effects on hematopoietic and mesenchymal stem cells when they are in a complex biological matrix also containing platelets. Experimental results indicate that stem cell proliferation at two weeks post treatment can be tuned as a function of electrical parameters. We demonstrate in this pilot study that stem cell proliferation can be either low (via conductive coupling, 8.5 kV/cm electric field amplitude) or high (via capacitive coupling, 2.5 kV/cm electric field amplitude) two weeks after stimulation, despite these two electric pulse delivery mechanisms inducing roughly similar growth factor release and immediate cell viability post treatment. These observations may open up additional ways of tuning electric pulse delivery for platelet activation and other biomedical applications.**

***Keywords—component; formatting; style; styling; insert** (key words)*

I. INTRODUCTION

Recent research has shown the exciting potential of ex vivo platelet activation via electric stimulation for potential wound healing applications in the clinic. The topical usage of activated platelet rich plasma (PRP), also termed as platelet gel, in wound healing has increased dramatically in the last decade. Clinical workflows for platelet gel applications involve: 1) blood draw from the patient (peripheral whole blood); 2) PRP separation from whole blood using centrifugation; 3) PRP activation typically via bovine thrombin; 4) topical application of activated PRP/platelet gel on the patient's wound. Activated PRP has a gel like consistency (due to clotting) and contains various growth factors released from platelets as a result of the activation process. Bovine thrombin has several drawbacks, such as relatively high cost, potential side effects (exposure of humans to bovine thrombin can stimulate antibody formation, potentially resulting in severe hemorrhagic or thrombotic complications) and the need for special storage conditions [1]. Platelet activation using electrical stimulation not only successfully addresses the drawbacks of bovine thrombin, but also presents a unique opportunity for greater standardization of this treatment process in the clinic.

Since whole blood and PRP are complex biological matrices with various cell types (red blood cells, platelets, white blood cells, stem cells etc.), it is critical to elucidate the impact of electric fields on other cell types, besides the platelets, present in PRP during activation via electric stimulation.

To answer this question, this work has focused on evaluating electric field effects on mesenchymal stem cells and hematopoietic stem cells, when these types of cells are present in a complex biological matrix containing platelets. Rather than quantifying growth factor release post electrical treatment in this pilot study, we assessed hematopoietic and mesenchymal stem cell growth by incubating cells in a suitable growth environment.

Hematopoietic stem cells (HSCs) mature into one of three types of blood cells: white blood cells, red blood cells, and platelets. While peripheral blood (that is in systemic circulation) contains some HSCs, the majority arise in bone marrow. Mesenchymal stem cells (MSCs) can differentiate into a number of cell types, including osteocytes, chondrocytes and adipocytes. While MSCs are found in several tissues, including fat and epidermis, they are also produced in the bone marrow. As a result of the colocation of HSCs and MSCs [2, 3], we used bone marrow aspirate to test stem cell response to electrical stimulation. More importantly, bone marrow can contain up to an 18-fold higher concentration of stem cells compared to peripheral blood [4]. Since peripheral blood contains some HSCs, but not MSCs, using bone marrow aspirate instead of peripheral blood enables us to test electric field effects on more

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stem cell types compared to the ones found in peripheral blood to expand the scientific investigation. The concentration of stem cells in bone marrow can vary dramatically based on multiple factors, such as age, donor health, and collection site. Stem cells represent only approximately 0.001% of mononucleated cells in bone marrow, which equates to approximately potentially 2000 colony forming units/mL of aspirate [5].

Section II outlines the materials and methods. Section III summarizes the experimental results. We make concluding remarks in Section IV.

II. Materials and Methods

We obtained 35mL of fresh human bone marrow aspirate from individual healthy donors (Lonza Walkersville Inc.). Upon receipt, each sample of bone marrow was analyzed (Sysmex XE-2100™) for initial cell populations. The remaining sample underwent standard density gradient centrifugation Ficoll (GE Healthcare) preparation to isolate the buffy coat – which contains mononuclear cells (MNC) and platelets. Following this separation, MNCs were counted (NUCLEOCOUNTER® NC-200™) to identify the number and viability of cells prior to further processing.

The isolated samples from each donor contained lymphocytes (T cells, B cells, and NK cells), monocytes, dendritic cells as well as hematopoietic progenitor and mesenchymal stem cells, with a total cell population of 16.8×10^6 cells/mL for the donor data presented here (this initial paper presents pilot study data obtained from processing and treating the bone marrow aspirate from one human donor; we plan to publish additional work including experimental trends from processing bone marrow aspirate from multiple donors). These isolated samples were then divided into four treatment conditions. We aliquoted samples into 0.5 mL commercial electroporation cuvettes with 2 mm electrode sampling and exposed them to two distinct electrostimulation pulse conditions referred to as Condition 1 and Condition 2, shown in Figs. 1a and 1b, respectively. The other two conditions were controls - buffy coat alone (BC) and buffy coat with bovine thrombin added (BT) - which were placed in 1.5mL centrifuge tubes. Neither BC nor BT controls received electrical pulsing. Buffy coat alone (no activator added) served as the negative control while buffy coat with bovine thrombin served as the positive control for this pilot test. BT was reconstituted in saline at

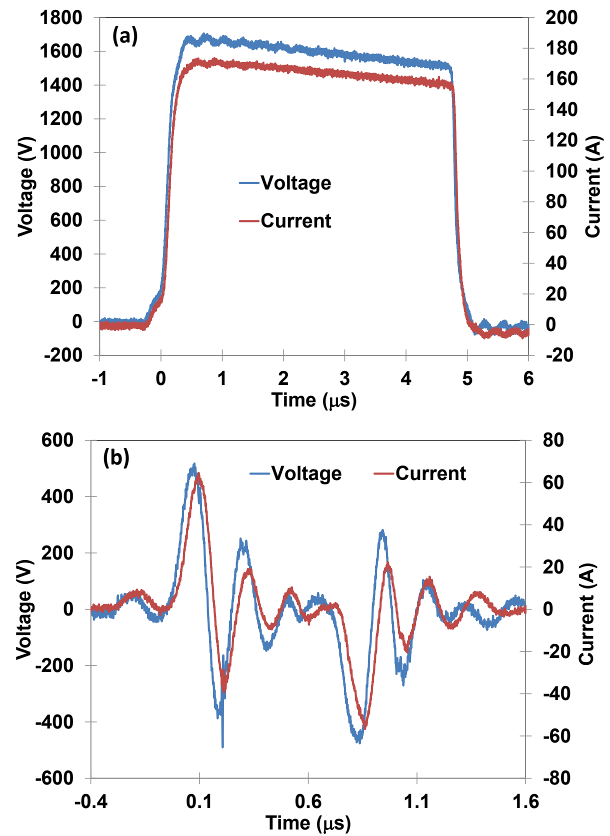

Fig. 1. (a) Current/voltage plot of electrical stimulation 1 (conductive coupling). (b) Current/voltage plot of electrical stimulation 2 (capacitive coupling).

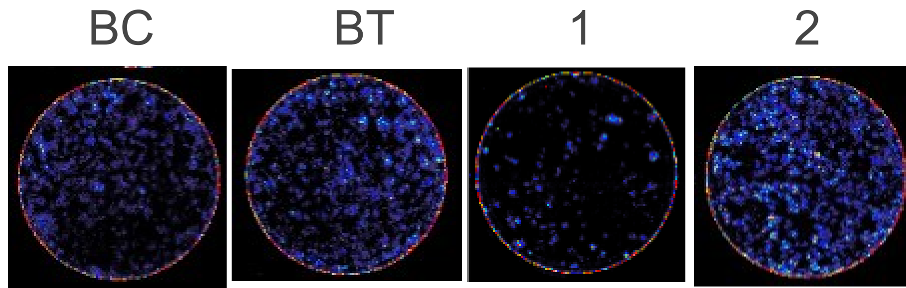

Fig. 2. Representative fluorescence of fibroblast populations stained with propidium iodide; BC – Buffy coat sample receiving no electrical pulsing, BT – Buffy coat sample treated with bovine thrombin but receiving no electrical pulsing, 1 – sample treated with electrical condition 1, 2 – sample treated with electrical condition 2. This method was used to assess mesenchymal stem cell proliferation post treatment. It is evident that electrical stimulation condition 1 has a negative effect on cell growth at two weeks (data for a single donor).

10U/μL. We added 50μL of this BT to 0.5mL of BC. Electrical stimulations 1 and 2 were previously evaluated in PRP activation studies, where PRP was separated via centrifugation from peripheral blood obtained from human donors [6]. These types of electrical conditions released relatively similar growth factor levels from PRP [6]. We achieved electrical condition 1 using conductive coupling – one monopolar pulse of high electrical field amplitude (8.5 kV/cm) and 5 μs duration. We achieved electrical condition 2 using capacitive coupling with eighty pairs of bipolar pulses of low electrical field amplitude (2.5 kV/cm) and approximately 150 ns duration. Reference 1 gives more details about conductive coupling implementation for platelet activation; capacitive coupling was achieved by introducing a 1.5 nF capacitor between the platelet activation instrument output and the cuvette with biological samples. The instrument is set to apply a square pulse of ~ 4 kV and about 500 ns duration, that translates into a bipolar pair of pulses due to the implemented capacitive coupling (see Figure 1b).

III. Experimental Results

We measured post-treatment viabilities of the isolated samples as 89 % (BC), 94 % (BT), 86 % (electrical condition 1), and 91 % (electrical condition 2). Practically, all samples have relatively similar viability – although it should be mentioned that this is "compounded" cell viability, for all cell types in this biological sample. To assess mesenchymal stem cell growth post electrical pulsing, cells were plated and incubated for two weeks after treatment. Periodic media changes selected for the adherent cell population, mesenchymal stem cells. Cells were then stained with propidium iodide and fluorescence intensity was measured on a Typhoon Imager to count colony outgrowths which permitted us to assess response to treatment by cell population (Fig. 2). The greater the signal, the higher the cell population.

Similarly, the growth of hematopoietic progenitor cells following electrical treatment was accomplished using a colony forming assay by mixing the cell population from cuvette or centrifuge tubes into Methocult (Stem Cell Technologies) and plating on grid plates for 14 days. A manual count of total colonies derived from the HSCs (colony and burst-forming units: BFU-E, CFU-E, CFU-GEMM, etc.) was performed (Fig. 3) using a standard light microscope. It is possible to identify BFU-E as they are easily recognized by their pinkish color, which is due to the presence of hemoglobin.

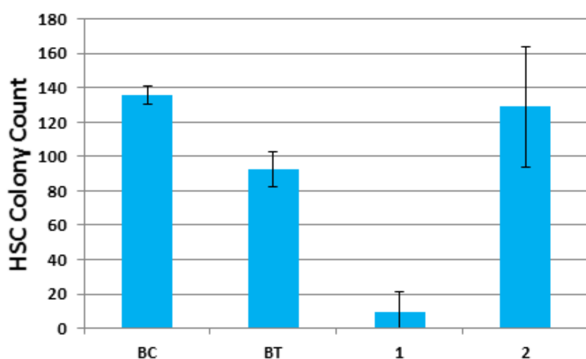

Fig. 3. Representative colony formation and growth of erythrocytes; BC – Buffy coat sample receiving no electrical pulsing, BT – Buffy coat sample treated with bovine thrombin but receiving no electrical pulsing, 1 – sample treated with electrical condition 1, 2 – sample treated with electrical condition 2. This method was used to assess hematopoietic stem cell proliferation post treatment. As in Fig. 2, it is evident that electrical stimulation condition 1 has a negative effect on cell growth at two weeks post plating (data for one donor).

IV. Conclusion

While further investigation is warranted, based on the procedures and limited data set described here, we have shown that electrical pulses that effectively stimulate platelet activation may not adversely affect stem cell proliferation post electrical treatment (e.g. condition 2). Moreover, these results tend to support the hypothesis that one could tune the effects on stem cells in platelet containing biological matrices by selecting specific electrical parameters that can preserve stem cell proliferation rates, while performing the desired platelet activation. Finally, we note that while cell proliferation is vastly different between electrical conditions 1 and 2 (low cell proliferation for condition 1 and high cell proliferation for condition 2 – as shown in Figs. 2 and 3) after two weeks, the cell viability measured immediately post treatment seems roughly similar for these two types of electric stimulation.

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