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The Influence of Pulsed Electromagnetic Fields on Embryonic Blood Vessel Development: Implications for Therapeutic Treatments

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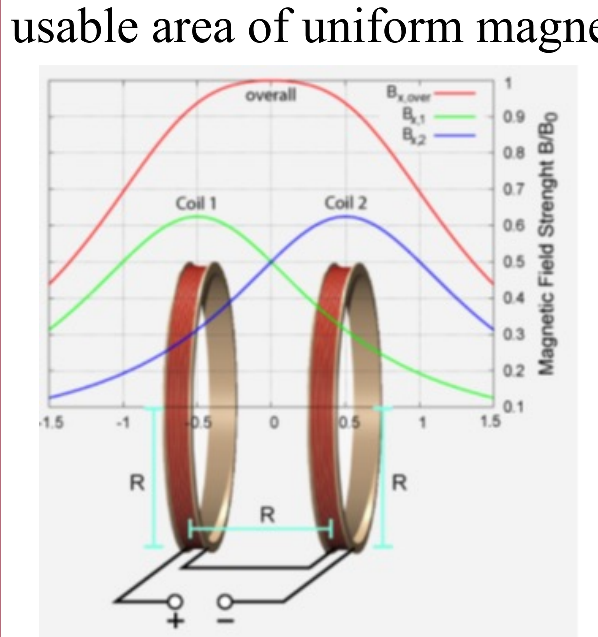
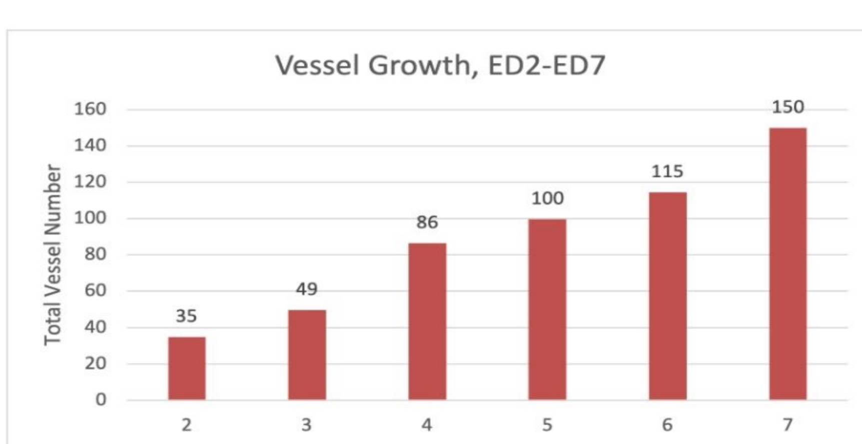
Abstract: This research delves into the intricate dynamics of blood vessel development under the influence of pulsed electromagnetic fields (PEMFs) during early stages of embryonic growth. Through careful examination and comparison between control and experimental groups, a myriad of developmental parameters was scrutinized, including but not limited to, blood vessel length, branching patterns, and the overall spatial area covered by vascular networks. A meticulously designed imaging regimen, spanning approximately a week per egg, ensures the acquisition of high-resolution images conducive to subsequent detailed data analysis. These images, captured for both control and experimental groups, undergo rigorous computational analysis, facilitated by public domain software (Image J, NIH). A plugin for this software, crafted specifically for vascular biology research, furnishes comprehensive daily developmental data for each egg, facilitating the identification of potential divergences in developmental profiles across an experimental time course. The discernible trends observed in our findings hint at a potentially beneficial effect of PEMFs on embryonic vascular development, surpassing those observed in the control group. These observations suggest promising implications for the advancement of PEMF technology as an "electro-pharmaceutical" treatment. Notably, the prospect of applying electromagnetic fields for noninvasive wound treatment represents a revolutionary leap in medical technology, promising transformative benefits for both human and animal healthcare. This study stands as a testament to the growing body of evidence underpinning the integration of electromagnetic wave-based therapies into mainstream medical practice, bolstering the basic research foundation for future advancements in this cross-disciplinary field.

Procedure: Each egg has a hole of diameter 38 mm cut into its top and is imaged each day to view the development of the embryo and its blood vessels. Each egg is imaged for 3-7 days, and the growth of its blood vessels undergo computational analysis using Image J software which provides details on growth parameters such as area covered by blood vessels, branch points, etc.

Two groups of eggs to go through the imaging process; the experimental group and the control group. In the control group, eggs develop in an incubator as they normally would, while for the experimental group eggs are placed in an incubator that is located within a Helmholtz Coil. This coil was used due to its simplicity, and the large usable area of uniform magnetic field it creates.





As data is collected for each group, we hope to find consistent growth charts for each egg as this given example. The growth charts of each egg will be compiled to compare results from the control and experimental groups. With this we hope to find experimental results showing the benefits of the use of EMFs.

Raw Experimental Data: Gaussian blurring and adaptive thresholding is utilized for image processing. A grey-scaling filter is applied to the image. Blurring is used to decrease random pixilated noise. A mathematical function then averages pixel values based on spatial positioning and intensity. Adaptive thresholding is dynamically applied to the different regions of the image, separating the features from the background, enabling the analysis software to process the image.

Figure 1

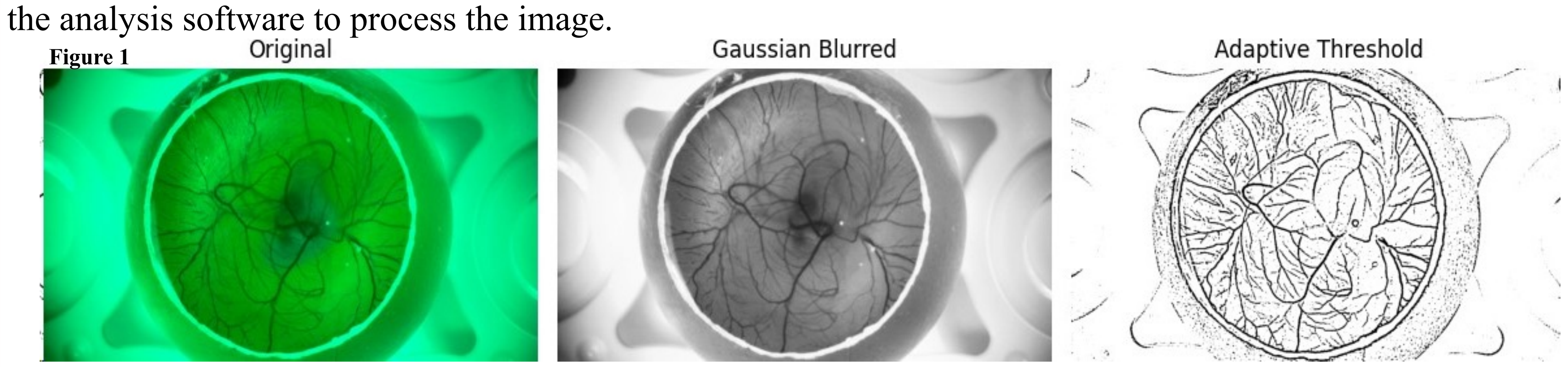


Figure 1: The processed images shown above are created for each egg, each day they are imaged. These images are used to collect data on the growth of the blood vessels within the embryo over the course of several days.

Experimental Findings: Given the images above for each egg, the image processing code collects the following parameters: *Total Blood Vessel Area, Vessel Density, Total Branch Points* and *Total Vessel Perimeter.* We found the most useful of the given parameters to be Total Blood Vessel Area (mm^2), which helps establish a consistent reading for the growth of the blood vessels during the embryo development. This data is recorded for each egg during each of its experimental days.

Experimental days refer to the system used to compare the eggs to each other throughout their development. All eggs start on "experimental day one" where the embryo is at an early point of development where there is viable data to be taken on that egg. This way, the eggs are compared during similar phases of development.

Data taken from 22 eggs (EMF v. Control Groups) over the course of four days is shown below.

Figure 2

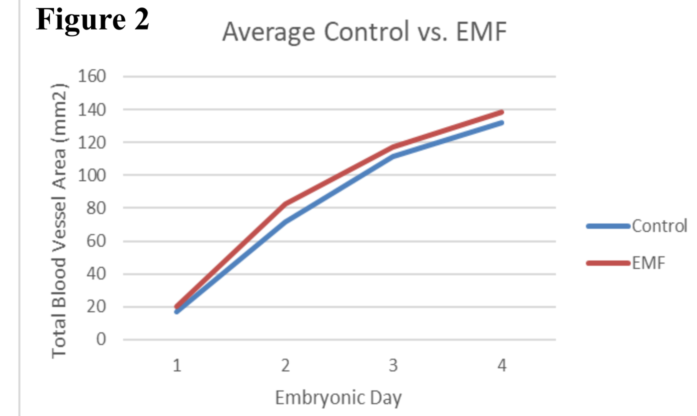


Figure 2: Average growth of control groups versus those treated with PEMF's. Overall increase in growth is shown in the PEMF group, however the increase is relatively small.

Figure 3

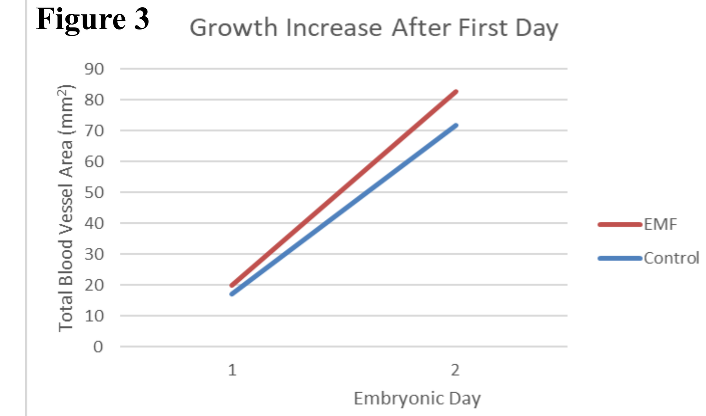


Figure 3: Largest increase in growth is seen in the first day of development.

Figure 4

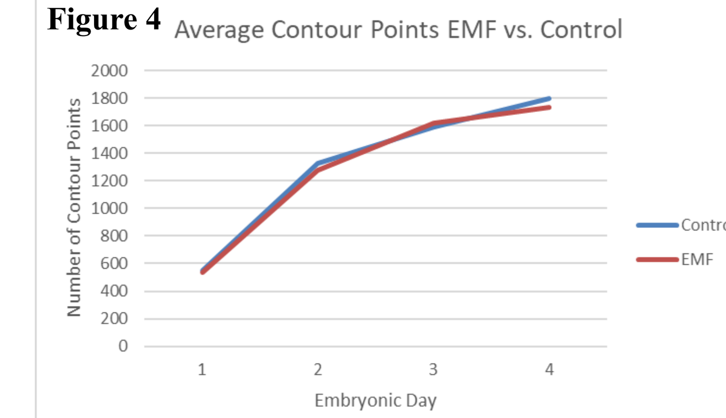


Figure 4: Average contour points serves to represent relative branching points between samples. Data follows similar trend to total blood vessel area.

Figure 5

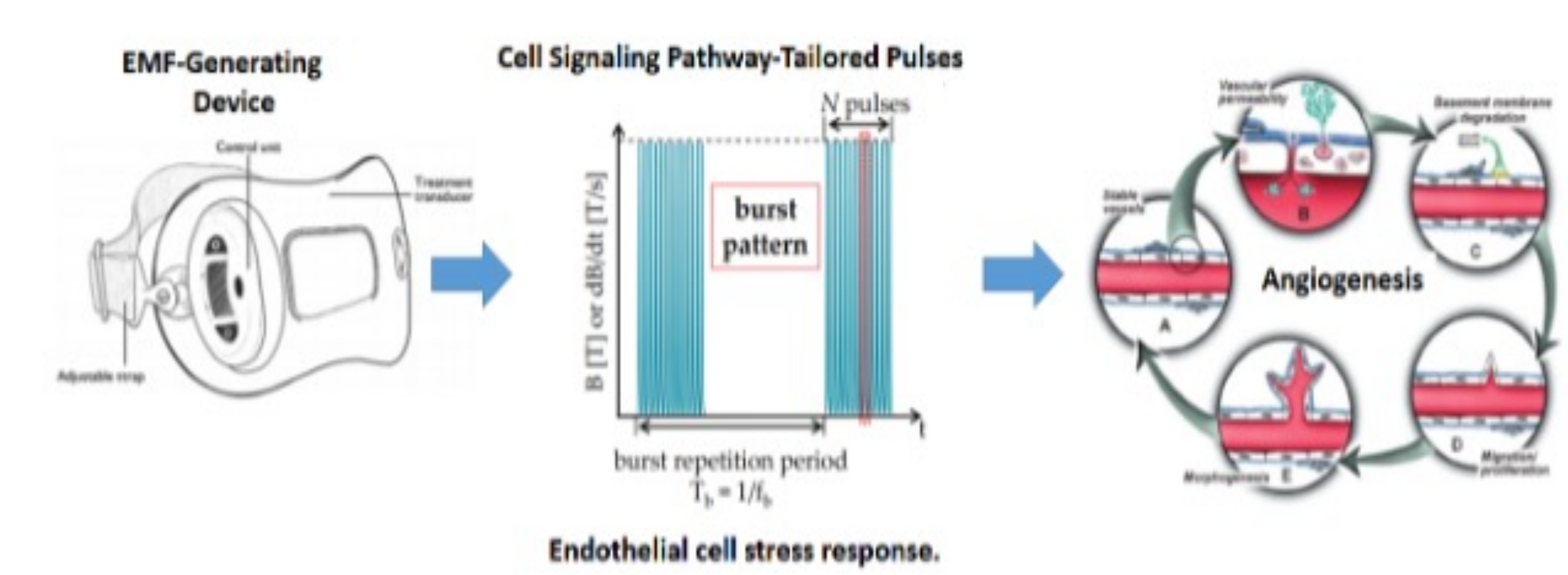


Figure 5: Possible implementation of PEMF device for patient use. Wearable devices would allow for easy use and application of PEMF therapy.

Experimental Findings for the Eggs: Evaluating the eggs individually rather than in an averaged experimental group, some trends in the development of the eggs can be ascertained. One trend is which experimental day each egg has shown the most development. Utilizing the examples given below and many other data recordings, one can determine when the eggs show more rapid signs of development.

Control Day 1	Control Day 2	Control Day 3	Control Day 4	EMF Day 1	EMF Day 2	EMF Day 3	EMF Day 4
21.79793248	89.51385657	107.448454	137.9602551	29.77213896	95.14530837	106.6085985	131.82489
24.29392394	91.19356747	126.4085591	161.935918	13.59976455	72.07433184	137.3708829	155.0667845
18.65657842	96.05294163	103.6116407	134.5477898	30.68271907	109.8442522	138.2136852	170.643893
10.04584992	45.2343198	96.7542946	105.1734771	20.04455005	65.37906314	114.391259	134.267838

Analyzing the data reveals that that the eggs are undergoing the most change between the first and second experimental days. The graph below shows the rate of blood vessel development between the first and second days.

Figure 6

Figure 6: The bar graphs show the blood vessel area coverage for each day of development. It is consistent that within both the control and EMF groups, in most the eggs we used to record data, the most blood vessel growth was observed between days 1 and 2, while some others may have grown faster between days 2 and 3.

Future work: Eggs for this study were obtained locally (Martin's Farm Supply, Potsdam, NY). Barnyard mixes of fertile brown chicken eggs, ranging in weight from 40 to 60 grams each, were employed. The magnitude of changes in EMF-induced vessel growth were generally smaller than the egg-to-egg variability. Future studies should use more expensive single variety eggs from a biological supply company. In addition, this study investigated very small magnetic fields of 1.3 mT. Higher field strengths can be applied to better differentiate PEMF-induced angiogenic responses.

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