New Applications of Non-Thermal Irreversible Electroporation of Tissues.

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Aim of Work:
The goal of this study is to introduce the fundamentals of non-thermal irreversible electroporation (NTIRE), the methodology of implementation and how applications are developed through this methodology. Because a significant and unique effect of NTIRE is on blood vessels, this will be the focus of the presentation. We will illustrate the methodology with new applications in: treatment of cancer, treatment of restenosis and in tissue engineering with NTIRE.
Materials and Methods:
A particular aspect of NTIRE is that it requires minimization or even complete elimination of any thermal effects so that the sole effect of the procedure is disruption of the cell membrane. To accomplish this goal NTIRE requires first treatment planning that needs to be later confirmed with histology. Treatment planning involves the simultaneous solution of the following equations with the appropriate tissue properties, boundary and initial conditions. The field equation for calculating the electric potential, ϕ: $\nabla\cdot\left(\sigma\nabla\phi\right)=0$ Resistive heating, P, (Joule heating): $P=\sigma\left|\nabla\phi\right|^2$
Bio-heat equation (Pennes) to calculate the temperature, T, as a function of time, t, in the presence of blood flow, ω_b, metabolic heating q, and Joule heating, P $\nabla\cdot\left(k\nabla T\right)+\omega_b c_b\left(T_a-T\right)+q+P=\rho c_p\frac{\partial T}{\partial t}$
Thermal damage expressed through an Arhenius energy activation integral as a function of the temperature, T, time of application, t, and activation energy, E: $\Omega(t)=\int_0^{\tau} Ae^{-\left[\frac{E}{RT}\right]}dt$
These equations were applied to tissues containing blood vessels with appropriately designed electrodes.
Sprague-Dewey rats and New Zeeland rabbits were used in these experiments in compliance with both the Principals of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, published by the National Institute of Health. After anesthesia, the left and right common carotid arteries were treated with a variety of procedures involving NTIRE of the carotids using typical NTIRE electrodes of the type described in Fig 1 and a high voltage pulse generator for electroporation procedures (ECM80, Harvard Apparatus, Holliston, MA).

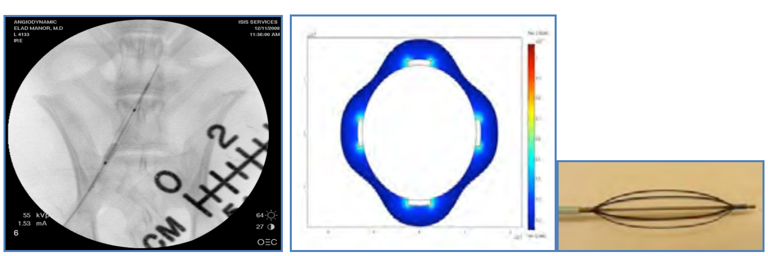

Figure 1: from right, four electrode NTIRE catheter, calculated electric field in a rat carotid artery, fluoroscopy of the catheter inserted in a rat carotid.

Results and Discussion:

The NTIRE protocols were designed first using the equation described above and results of the type in Fig 1 (middle) were used to choose the appropriate protocol. Figure 2 illustrates a result relevant to restenosis and shows the difference between a rat carotid artery treated with NTIRE after angiography and one that was not treated with NTIRE, 28 days after the procedure. It is evident that NTIRE can inhibit restenosys.

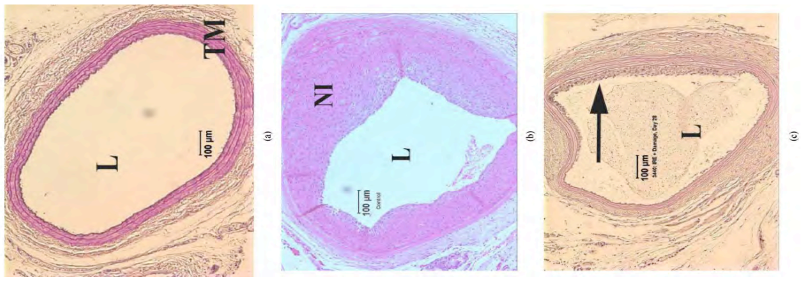

Figure 2: Cross section through the lumen (L) of a carotid artery of a rat. Left: normal, middle 28 days after angioplasty – note the neointimal formation (NI), right NTIRE treatment after angioplasty – note minimal NI (arrow)

The second new application deals with the idea that the molecular selectivity of NTIRE can be used for tissue decellularization in a way that preserves the extracellular scaffold intact. Figure 3 shows that a week after NTIRE the carotid artery is completely decellularized – but the extracellular scaffold is sufficiently intact to facilitate regrowth of endothelial cells. Our results show that if harvested after three days the decellularized artery can be used as a scaffold for tissue engineering.

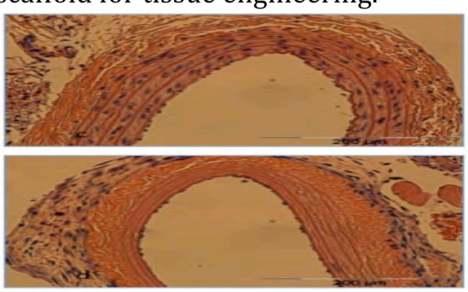

Figure 3. cross section through the carotid artery: left normal – right one week after NTIRE

Conclusion:

NTIRE is a new application of pulsed electric fields in a mode that affects only the cell membranes and keeps all the other molecules, in particular the extracellular matrix intact. This makes possible the treatment of cancer near critical tissue structures, such as in the pancreas and the treatment on blood vessels such as for treatment of restenosis or for producing a scaffold of the extracellular matrix for tissue engineering.