Production of Hybridomas by Electrofusion

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The technique of antigen-directed electrofusion[1] offers several advantages in the production of hybridomas for monoclonal antibody secretion. First, a larger proportion of the hybridomas produced by the technique secrete antibodies against the immunizing antigen. This saves time and resources used in screening large numbers of hybrids that produce irrelevant antibodies, or no antibodies at all. Second, the technique insures that an increased number of cells that fuse are those expressing antibodies of higher affinity for the antigen, and therefore a greater proportion of the antibodies arising are useful for immunochemical techniques.

We have previously reported the use of an electroporation apparatus to provide the electrical pulse necessary to induce cell fusion.[2] In the paper, we made the claim that the technique could be applied to a commercially available apparatus, providing that the system was first calibrated by looking at the effects of the pulses on myeloma cells alone. The conditions that result in the killing of approximately 40–60% of myeloma cells appear to be effective in causing fusions between myeloma cells and mouse splenocytes.

The Gene Pulser® apparatus was tested for efficiency of hybridoma production. This unit generates an exponentially decaying pulse, the length of which is regulated by a series of internal capacitances. Figure 2 shows the survival of mouse NS-1 myeloma cells under a range of pulse conditions. The electrical pulses were applied in 0.4 cm electrode gap cuvettes in an unmodified Bio-Rad slide and sample chamber (not incorporating the resistors described in our previous publication). Two antigen-directed electrofusion experiments were then carried out as previously described,[2] using wool keratin low sulphur protein and a synthetic peptide homologous to a plant virus coat protein sequence, coupled to bovine serum albumin as antigens. The results are shown in Table 1. It can be seen that the proportion of antibody secreting hybrids obtained with the Gene Pulser apparatus was close to 100%. The pulse conditions chosen gave approximately the same numbers of hybrids, but 400 V and 1 µF for 2 pulses appeared to be the most efficient.

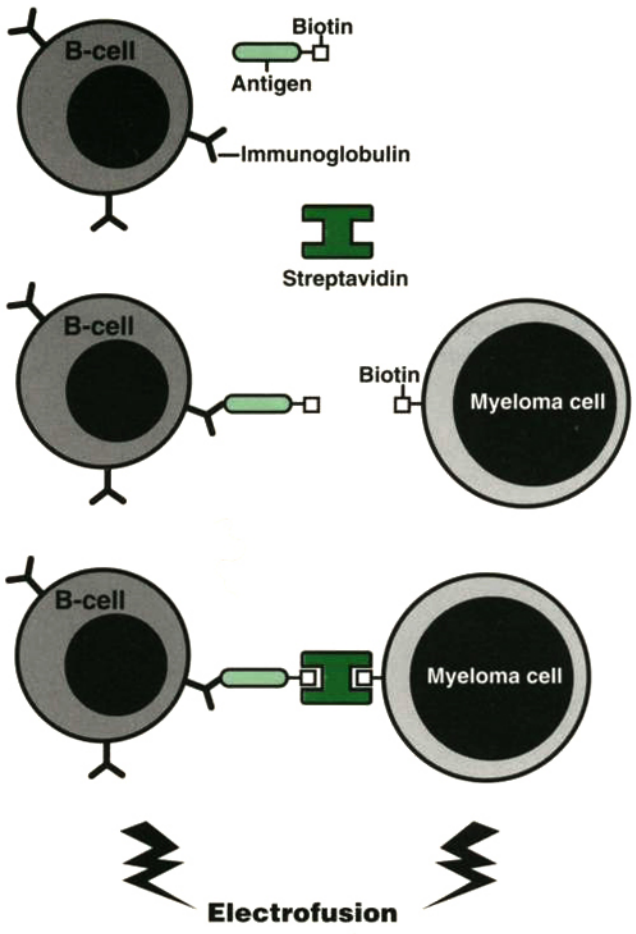
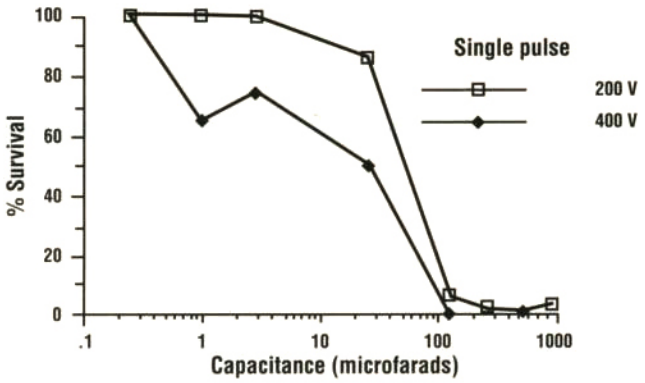


Fig. 1. Schematic representation of antigen-directed electrofusion.

We conclude that the Gene Pulser apparatus is suitable for performing this technique, and, because of its simple electrical pulse shape, it is less expensive and more convenient to use than other types of instruments specifically designed for mammalian cell fusion.



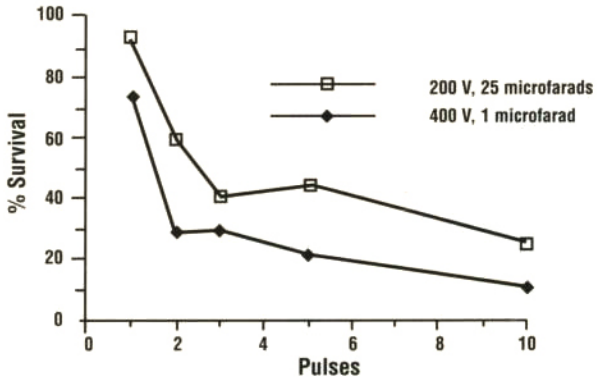


Fig. 2. The survival of mouse NS-1 myeloma cells under various pulse conditions.

Table 1

	Condition 1	Condition 2	Condition 3
Voltage (V)	200	200	400
Capacitance (μF)	25	25	1
Number of pulses	2	3	2
Antigen:			
(1) Wool keratin hybrids	52	55	81
% Positive for antigen	100%	100%	100%
(2) Plant virus peptide hybrids			41
% positive for antigen			100%

References

1. Wojchowski, D. M. and Sytkowski, A. J., Hybridoma production by simplified avidin-mediated electrofusion, *J. Immunol. Meth.*, **90,** 173 - 177 (1986).
2. Hewish, D. R. and Werkmeister, J. A., The use of an electroporation apparatus for the production of murine hybridomas, *J. Immunol. Meth.*, **120,** 285 - 289 (1989).

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