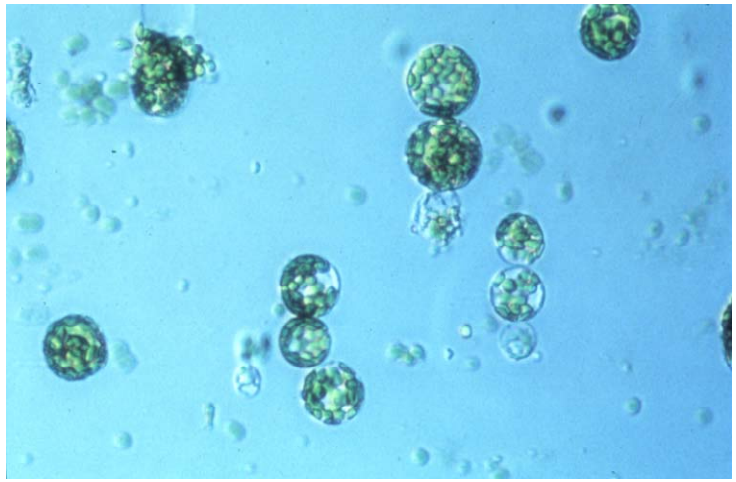
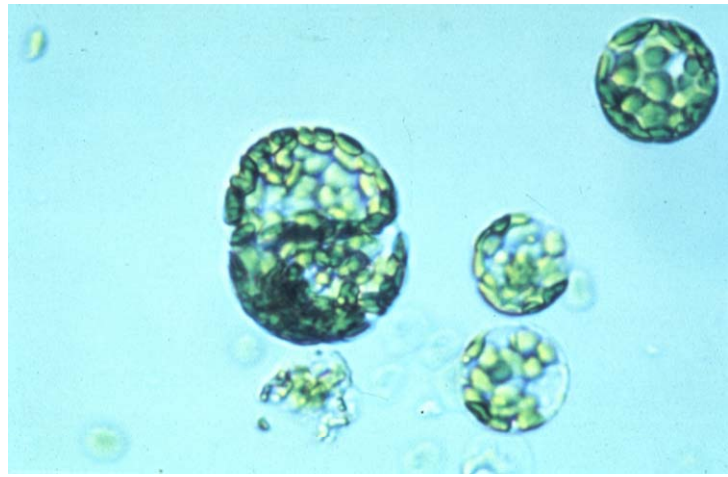
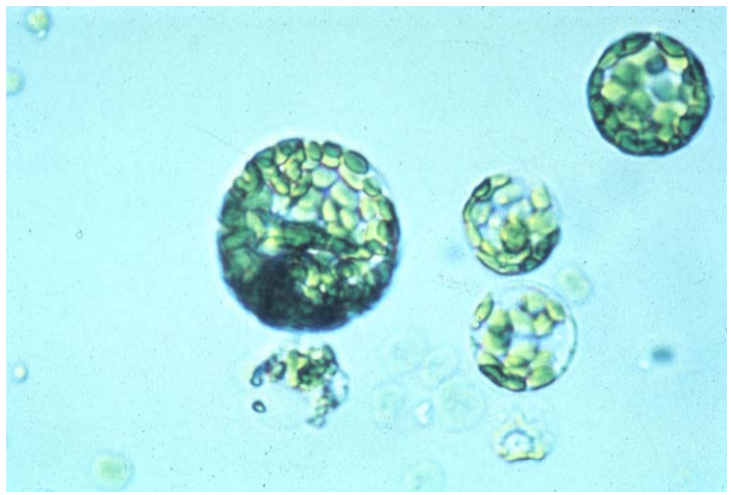
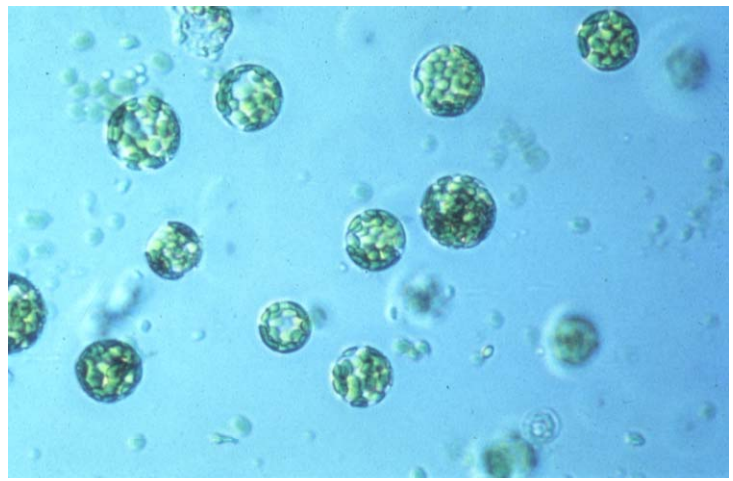
Electrofusion vs PEG









Cell fusion Applications

Major Applications

Nuclear Transfer
The nucleus of one cell is transferred to the cytoplasm of another cell (usually an unucleated oocyte)

Hybridoma Production
Antibody secreting B cells are fused to immortalized myeloma cells

Tumor Vaccines
Dendritic cells are fused to tumor cells to produce a specific vaccine against a tumor

How does **P**oly-**E**thylene **G**ycol-mediated fusion work?

The mechanism by which PEG fuses cells is not well understood. Some theories include:

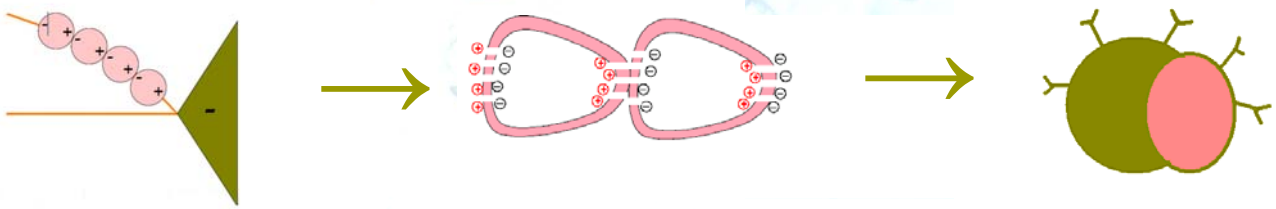
1. PEG can bring vesicle membranes to near molecular contact by making the water between two cells thermodynamically unfavorable. A disruption in packing of the contacting monolayers of the membranes is needed for fusion to occur.[7]
2. Dehydration leads to asymmetry in the lipid packing pressure in the two leaflets of the membrane bilayer leading to formation of a single bilayer septum at a point of close apposition of two cell membranes. The single bilayer septum then decays during formation of the initial fusion pore. Agents that enhance or alleviate the dehydration-induced asymmetric packing stress will favor or inhibit fusion.[8]

How does electrofusion work?

Electrofusion joins the membranes of neighboring cells by the application of a pulsed electrical field. Electrofusion uses the properties of two waveforms; an oscillating AC waveform for cell alignment, and a DC square wave pulse for the fusion:

1. The AC waveform is applied to align cells by dielectrophoresis. More specifically, an electric field induces a dipole within cell. As cells move toward a common point the dipoles attract and pearl-chain formation results.
2. A DC waveform pulse is applied which fuses the cells together whereby a brief but intense electric field forms temporary pathways or pores in the cell membrane.
3. A post fusion AC waveform pulse is applied to hold fused cells together while they mature, the waveform holds cells in place with gentle force to promote fusion.

EASY AS 1 – 2 - 3



How does PEG compare?

PEG

- Peroxide build up in PEG solutions contributes to cell cytoxicity.[2]
- Aldehydes can build up in PEG due to autoclaving or non-optimal storage conditions.[2]
- PEG is less effective at promoting fusion when cells are in suspension versus cells that are attached to a substrate[2]
- Lower fusion efficiencies[3]
- $<10^6$ cells are required for PEG-mediated fusion[6]
- PEG-induced fusion is unreliable since the success of a fusion depends on many variables such as the size and shape of the pellet and method by which PEG is stirred into the resuspended cell pellet[3,4,5]
- The actual method by which PEG works is not fully understood[1]
- Cell membranes undergoing fusion with PEG are uniformly affected, which may cause a greater loss of cellular constituents[6]

ELECTROFUSION

- Considerably higher efficiencies for many cell types – Hybrid yields are up to 80-fold over PEG-mediated fusion[5]
- Better reproducibility
- Significantly lower amount of B cells required
- Fast and easy-to-use protocol, multiple fusions can be performed in a short period of time
- Better growth properties in the early stage following fusion[2]
- Optimized and reproducible protocols are available for specific cells
- Direct control of fusion results
- Controllable physical parameters that are independent of genetic, biochemical, physiological, or morphological cell properties[5]
- Simple Technique
- Can be viewed in Real-Time under a microscope

Experimental Data

Based on data provided from: *Orentas, R. et al*

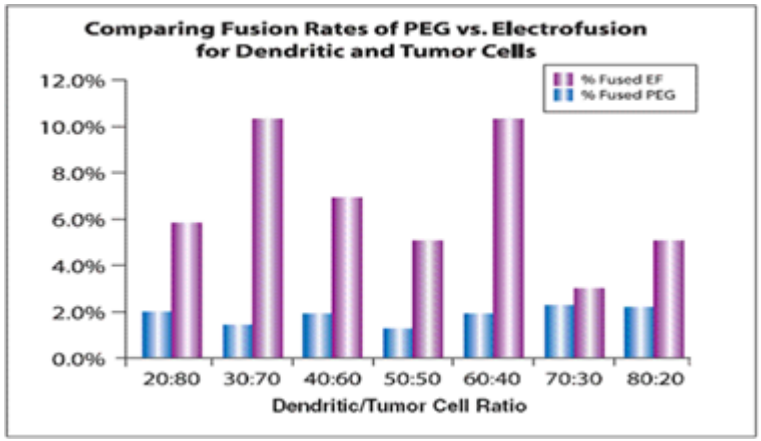


Fig 2 HMMA 2.5 cells were fused with EBV-transformed human B cells using previously determined optimal electrofusion parameters to test the effect of optimized cell treatment conditions before and after electro- fusion, as detailed description in the results section. Electrofusion also was compared with PEG fusion. The cell ratio in the PEG fusion was 1:3 (partner cells : transformed B cells) and 2:1 in electrofusion. The input number of EBV transformed B cells was 3 × 10^6 in all experiments. Hybridoma selection and colony counting were performed in the same way as previous experiments. The columns represent the average fusion efficiency (%) from two experiments; error bars represent the SD.

Yu, X. et al Human Hybridomas

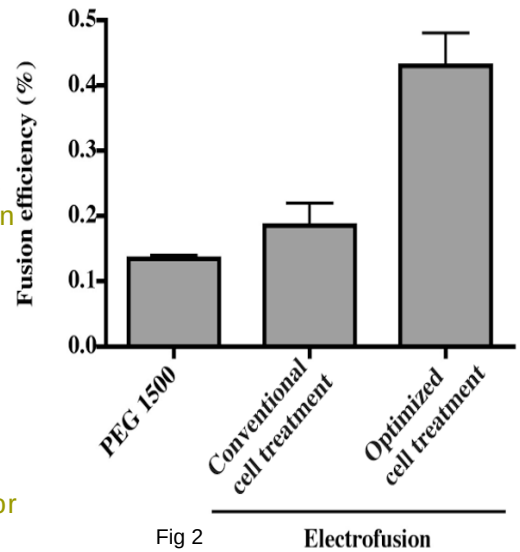


Fig 2

B Lymphocytes fused to myeloma cells Fig 3

Experiment Number	Antigen Specific Clones	
	E-Fusion	PEG
1	20	0
2	10	0
3	400	23
4	151	21
Mean	145	11

Fig 3 An example of E-fusion versus PEG was produced by M. Coccia, Ph.D., Platform Development Group at Medarex, Inc., Milpitas, CA.: Transgenic human Ab producing mice were used in experiments comparing efficiencies of E-fusion to PEG fusion. Each experiment used a portion of the same splenocyte preparation from mice immunized with tetanus toxoid (TT) for comparison of the E-fusion and PEG fusion methods. Results shown are the number of TT antigen-specific clones generated by each method normalized to the same number of cells. These data are representative of additional experiments (not shown) utilizing four different antigens. Taken together, all experiments showed E-Fusion generated approximately ten-fold more antigen specific antibody clones relative to PEG fusion.

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Summary

- Electrofusion is more efficient than PEG
- Results are reproducible
- Reliable process
- Electrofusion is more cost effective
- Optimized protocols have been established

References

[1]http://www.umass.edu/vetimm/docs/Wagner_Hybridoma.pdf

[2]Bartal, A. et al.,1987, *Methods of Hybridoma Formation*

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[4]Karsten, S. et al., Direct comparison of electric field-mediated and PEG-mediated cell fusion for the generation of antibody producing hybridomas, 1988, *Hybridoma 7*, 627-633

[5]Radomska, H. et al., Mammalian Cell Fusion in an Electroporation Device, 1995, *Journal of Immunological Methods*

[6]Kreutz, F. T. et al., A New Method to Generate Quadromas by Electrofusion and FACS Sorting, Vol 17, No 3, 1998, *Hybridoma*

[7]Lentz, BR. Et al., Poly(ethylene glycol) (PEG)-mediated fusion between pure lipid bilayers: a mechanism in common with viral fusion and secretory vesicle release? 1999, *Molecular Membrane Biology*

[8]Lentz, Br et al., Polymer-induced membrane fusion: potential mechanism and relation to cell fusion events, 1994, *Chem Phys Lipids*

[9]Yu, X. et al., An optimized electrofusion-based protocol for generating virus specific human monoclonal antibodies, 2008, *Journal of Immunological Methods*