Characterization of mediator-less sugar/oxygen enzymatic fuel cells *in vitro*

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Biofuel cells (BFCs) constitute a subset of fuel cells (FCs) that employ biocatalysts instead of the typical non-biogenous catalysts (1). The enzymatic fuel cells (EFCs) based on three-dimensional nanostructures loaded with enzymes represent one of the most compact BFC designs (2). The study aimed to investigate the performance of a membrane less, mediator less sugar/oxygen EFC *in vitro*.

An EFC with *Corynascus thermophilus* cellobiose dehydrogenase (*Ct*CDH) as bioanode and *Myrothecium verrucaria* bilirubin oxidase (*Mv*BOx) as biocathode enzymes was constructed at the bottom of an ECIS medusa cell culture plate. The surface of planar electrodes on the bottom of culture plates were modified by drop depositing gold nanoparticles (AuNPs). The resulting 3D structure was loaded with the appropriate enzyme. Then L929 murine fibroblast cells were seeded on top of the EFC and the effects of the EFC on the cells and vice versa were studied.

It was found that on average the power of the EFC drops by about 70% under a nearly confluent layer of cells (Fig. 1). In summary, the L929 cells can consume oxygen, restrict diffusion of lactose to bioanode and/or degrade *Ct*CDH and *Mv*BOx by extracellular proteases. All these factors can affect EFC stability to a certain degree.

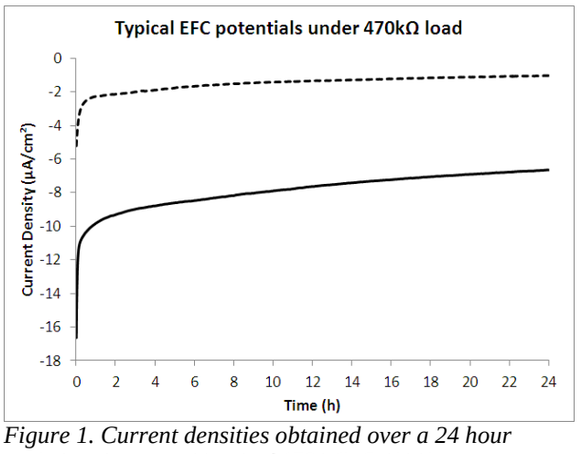

Figure 1. Current densities obtained over a 24 hour period under a workload of 470 kΩ. Solid line represents the EFC in cell medium with 5 mM lactose and no seeded cells. Dashed line is the EFC in cell medium with 5 mM lactose under a nearly confluent layer of L929 murine fibroblast cells.

The EFC appeared to have a toxic effect on the L929 cell line. It was concluded that the bioanode enzyme, *Ct*CDH, most likely produced small amounts of hydrogen peroxide (3, 4), which is toxic to cells. The toxic effect could be completely circumvented by co-immobilizing catalase on the bioanode (Fig. 2).

These results demonstrate the approach on how EFCs could be tested *in vitro* for possible toxic effects and tissue damage. This is especially important if such a device is considered for implantation.

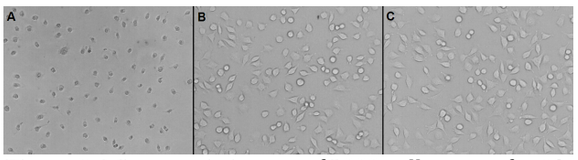

Figure 2. Microscopic images of L929 cells grown for 18 hours in between the electrodes of medusa cell culturing plate. The cell medium contained 5 mM lactose. **A:** *the electrodes on the plate were modified to provide EFC.* **B:** *EFC anode was made by co-immobilizing catalase.* **C:** *was a control, i.e., the electrodes in medusa plate were left without modification.*

References

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