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ENHANCING THE SENSITIVITY OF THE SEPTIC BACTERIUM DETECTION METHOD BY CONCENTRATING THE PHAGE-INFECTED BACTERIA VIA DC ELECTRICAL CURRENT [1]

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In this Letter, we propose a simple method to strongly enhance the sensitivity of the detection of the new technique called "SEnsing of Phage-Triggered Ion Cascade (SEPTIC)". The method accumulates those phage infected bacteria which are emitting ions and releases those which have stopped the ion emission activity. The estimated increasing of the sensitivity can reach several orders of magnitude at practical conditions.

Keywords: Prompt identification of bacteria; detection limits; nano-bio chip; phages; noise.

1. The New Method: Infected Bacterium Collection by DC Current

Recently, a new bacterium detection and identification method "SEnsing of Phage-Triggered Ion Cascade (SEPTIC)" has been proposed [1,2] which is able to identify living bacteria within a few minutes time with unparalleled specificity. The method utilizes the fact that, after phage infection, the bacteria emit about 10^8 ions into the ambient fluid and the voltage fluctuations induced by this ion emission can be detected with two thin metal film electrodes of a size of a few microns. In this Letter we propose a simple tool to strongly enhance the sensitivity of the method.

The Debye length (electrostatic screening length) in electrolytes is well below micron. Any static electrical field gets screened with an exponentially decaying tail beyond the Debye length. That fact indicates that the physical conditions in electrolytes are not favorable if the ionized object is not in the direct vicinity of the detecting electrodes. Let us consider a square shaped thin film electrode of 10 micron size. If the concentration of bacteria is 10^6 bacteria/mm^3, which is the experimental conditions in [1], then on the average there is 1 bacterium in a 10 micron size cube, so we have only one bacterium in

[1] The content of this paper is the subject of a Texas A&M University patent disclosure dated May 18, 2005.

the vicinity of the electrode and that is not a direct vicinity because its mean distance from the electrode is several microns. Therefore, at this condition, a very low ionic signal, order of magnitudes less than at direct touch, is expected because the electrical field decays exponentially fast beyond the Debye length.

However, if a dc current is forced through the electrolyte between the two electrodes then the situation radically changes. Then the Debye theory is not applicable and a steady long-range electrical field will expand through the electrolyte. This electrical field will be able to draw the charged (infected) bacteria to the electrodes where they can be in a direct touch with the surface. The great advantage of the method that it attracts only those bacteria which are active (emitting ions) regarding the SEPTIC detection method. As soon as a given bacterium finished its ion emission and its excess charge gets neutralized, its interaction with the electrical field stops and it will diffuse away from the electrode.

2. Physical Analysis and the Viability of the Method

Though the details of the dynamics and charge distribution of the ion emission of bacteria is unknown, simple physical considerations allow some crude estimations about the effectiveness of this enhancement of the sensitivity of the SEPTIC technique. The diffusion coefficient, diffusion time and equilibrium ionization number was already mentioned in [3] and the rest of the considerations are new.

The diffusion coefficient of a sphere with radius r in a fluid with viscosity η is given by the Stoke's law.

$$D_s = \frac{kT}{6\pi\eta r} \ . \tag{1}$$

For a spherical bacterium with diameter of 1 micron, the diffusion coefficient in water is

$$D_B \approx 4.4 * 10^{-13} \ \left[\frac{\mathrm{m}^2}{\mathrm{s}}\right]. \tag{2}$$

Thus, taking the above example, the diffusion time through the 10 micro size cube (see above) is (see also [3]):

$$\tau_B = \frac{L^2}{D_B} = 227 \ [\mathrm{s}] \ . \tag{3}$$

Because in this practical example, on the average there is only one bacterium in a 10 micron size cube, this result means that, without dc current, during the typical duration (120 s) of a SEPTIC measurement, we can measure ions only from a single bacterium. At lower concentrations, we may not even be able to get any bacterium signal.

Let us study the electrical transport properties of the charged bacteria. They emit 10^8 ions after the phage infection, however their net charge is strongly limited by the Coulomb energy. If the bacterium is charged up by $Z_B q$ where q is the elementary charge and Z_B is the ionization number, then the total energy needed to it can be estimated as:

$$E_{Cb} \approx \frac{1}{4\pi\varepsilon\varepsilon_0}\frac{Z_B^2 q^2}{r}. \tag{4}$$

If we want to add more charge and increase the ionization number by ΔZ_B, the energy needed to this can be approximated as follows:

$$E_{\Delta Z} = \frac{dE_{Cb}}{dZ_B}\Delta Z_B = \frac{1}{4\pi\varepsilon\varepsilon_0}\frac{2Z_B q^2}{r}\Delta Z_B \ . \tag{5}$$

Thus, in the above example of a micron size bacterium, to increase the ionization number by one, we need the following energy:

$$E_1 = \frac{1}{4\pi\varepsilon\varepsilon_0}\frac{2Z_B q^2}{r} \approx 10^{-23} Z_B \ \left[\mathrm{J}\right]. \tag{6}$$

First, let us suppose a thermally activated ionization process, which is the lowest limit relevant to the actual situation. Then

$$E_1 \approx kT \approx 4 * 10^{-21} \ \left[J\right], \tag{7}$$

thus [3]:

$$Z_{\max,eq} \approx \frac{kT 4\pi\varepsilon\varepsilon_0 r}{2q^2} \approx 812. \tag{8}$$

However, which was not considered in [3] is the fact that driven nonequilibrium systems do not have this limitation so the actual ionization number may be much larger. To reach a crude estimation, let us suppose that the ion emission is driven by an electrochemical force with a characteristic energy of 1 eV which we consider as an upper limit. Then,

$$E_1 = 1.6 * 10^{-19} \ \left[J\right] \tag{9}$$

and

$$Z_{\max,eq} \approx \frac{E_1 4\pi\varepsilon\varepsilon_0 r}{2q^2} \approx 31400. \tag{10}$$

In conclusion, the maximum ionization number of bacteria with 1 micron size is in the range of 1000 - 30,000.

Now, let us estimate the electrical transport properties versus the ionization number which is a new issue. The application of the Einstein equation between the mobility and the diffusion constant yields:

$$\mu_B = \frac{Z_B q D_B}{kT} \ . \tag{11}$$

The drift velocity of charged bacteria in a dc electrical field E_{dc} is:

$$u_d = \mu_B * E_{dc} = \frac{Z_B q D_B}{kT} E_{dc} . \tag{12}$$

At 1 V/m electrical field with a 1 micron size bacteria charged to 10,000 q

$$u_d \approx 1.8 * 10^{-7} \left[\frac{\mathrm{m}}{\mathrm{s}} \right]. \tag{13}$$

At 1000 V/m electrical field, which is easy to make at the length scale of 100 microns by a voltage of 0.1 V, the drift velocity is 0.18 mm/second, so all bacteria in the 100 micron length range would be collected at the electrodes within less than a second. This calculation indicates that, the bacteria can be drifted through the fluid and collected at the electrode with a high speed.

The current density of bacterium number is:

$$J_{Bnumber} = n_B u_d = n_B \mu_B E_{dc} = \frac{Z_B q D_B n_B}{kT} E_{dc} \tag{14}$$

If the dc current I is dominated by the background ion current in the fluid, then:

$$E_{dc} \propto I_{dc} . \tag{15}$$

That is the bacterium current density will satisfy:

$$J_B \propto I_{dc} . \tag{16}$$

The estimation of the signal enhancement is not easy without exactly knowing the mechanism of the generation of the ion cascades and the possible influence of electrical field on that. However, a simple estimation can be made by using the fact that the observed fluctuations have $1/f^2$ type power density spectrum. Then the acceleration of the bacterium flow with increased currents can contribute to an accelerated fluctuation. A recorded $1/f^2$ noise with spectrum A/f^2 will have a spectrum of k^2A/f^2 if it is played back with a speed increased by a factor of K because of the $f ==> Kf$ transformation. Thus, from the above argumentation, we expect that the spectrum will scale as:

$$S_{u,accelerated}(f) \propto J_B^2 \propto I_{dc}^2 . \tag{17}$$

On the other hand, there is more than just playing the fluctuations faster at increased currents. The electrodes will accumulate a larger number of bacteria within the lifetime of ion cascades which implies also an increased amplitude of the fluctuations due to cross-correlation effects. If this increase scales linearly with the bacterium current density J_B that effect will also result in a scaling of the power density spectrum by I^2. Then, due to the two separate effects, we get:

$$S_u(f) \propto J_B^4 \propto I_{dc}^4 . \tag{18}$$

However, because the two effects outlined above may be two sides of a single physical mechanism, thus they may not be independent or; in other cases the space charge of the bacteria the second effect may saturate at a certain current density, frequency and after a characteristic time of saturation, thus it is proper to conclude the estimation as:

$$S_u(f) \propto I_{dc}^X \tag{19}$$

where $2 \le X \le 4$.

It is important to note that the scaling/enhancement of the power spectrum described by Eq. 19 is relevant for the already increased signal due to the removal of the Debye screening (see above) because the "active" bacteria are touching the electrode when there is a dc current. If we suppose a Debye screening length of 0.25 micron and, in the case of zero dc current, an effective distance of 3 microns of the bacterium from the electrode, then the dc current situation with the bacterium touching the electrode provides about $\exp[3/0.25] \approx 15,000$ times enhanced electrical field.

It is also obvious that the amplification based on removing Debye screening works with full strength only up to the first layers of bacteria over the electrode because the next layers will suffer increasing Debye screening.

Finally, this simple picture suggests that, in the case of a fixed current and electrode sizes, an increasing gap size between the electrodes will result in an increasing efficiency of this enhancement technique.

3. Conclusion

Even from these crude considerations with limited accuracy, it is obvious that the proposed technique of feeding the electrodes by a dc current generator, and so letting a dc current through the electrolyte, can increase the sensitivity of SEPTIC by several orders of magnitude. Due to its effectiveness and simplicity, it is hard to imagine that any practical SEPTIC applications could avoid using this technique. The results indicate that the gap size does not have to be in the nanometer range and a larger gap size can make enhancement more efficient.

Finally, it is important to note (due to a relevant comment of A. Der) that the present approach/calculations are strongly simplified and taking into the account Debye's electrophoresis theory can significantly refine them.

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