**Appendix**

**Calculation of the mechanical force in electro-piercing:**

We derive the mechanical force required to generate a 100 m deformation with a needle electrode by applying Tan’s medaka fish egg mechanical piercing model. The properties of medaka fish eggs, the needle-electrode and the glass capillary are listed in Table II. To calculate the Hertzian stress *M\_100* on the needle-egg contact region, the tip of the needle electrode (outer diameter 70 m, inner diameter 50 m, and apical angle 40°) is simplified as a sphere with the wall thickness of the needle.

The stress to produce a 100 m deformation with a glass capillary (inner diameter *DOI=14 mm*, outer diameter *Do= 25 m*) and a force *FM\_100*, could be calculated as:

(A-1)

The force *FE\_m* to generate *M\_100* with a needle electrode and fish egg is computed by Hertzian contact mechanics[[57](#_ENREF_57)], where *λ, E, d*, represent the Poisson ratio and the Young’s modulus, respectively. The subscript *n* and *e* represent the needle and the fish egg, respectively.

　　 （A-2）

Therefore, the total force for electro-piercing, *FE*, i.e., the summation of the mechanical force *FE\_m* and the electric force *FE\_e*（3 N-82 N）is between 245 N and 325 N.

The maximum contact stress generated by the total force during electro-piercing, *E\_max,*  is:

(A-3)

The contact stress required to puncture the fish egg by mechanical piercing is calculated by the puncture force *FM\_Max* and the contact area:   
　 (A-4)

TABLE II. Properties of medaka fish eggs and piercing capillaries

|  |  |  |
| --- | --- | --- |
| Properties | Symbol | Value |
| Poisson ratio of nickel |  | 0.31 |
| Poisson ratio of a fish egg |  | 0.5 (incompressible) |
| Elastic modulus of nickel |  | 0.45 GPa |
| Elastic modulus of a fish egg |  | 2.83 MPa[[10](#_ENREF_10)] |
| Outer diameter of the glass capillary |  | 25 m[[10](#_ENREF_10)] |
| Inner diameter of the glass capillary |  | 14 m[[10](#_ENREF_10)] |
| Wall thickness of the needle electrode |  | 10 m |
| Force to cause 100 mm deformation with a glass capillary |  | 1 mN[[10](#_ENREF_10)] |
| Force to puncture a fish egg with the glass capillary |  | 12.5 mN[[10](#_ENREF_10)] |