Adherent primary cultures of mouse intercostal muscle fibers for isolated fiber studies.

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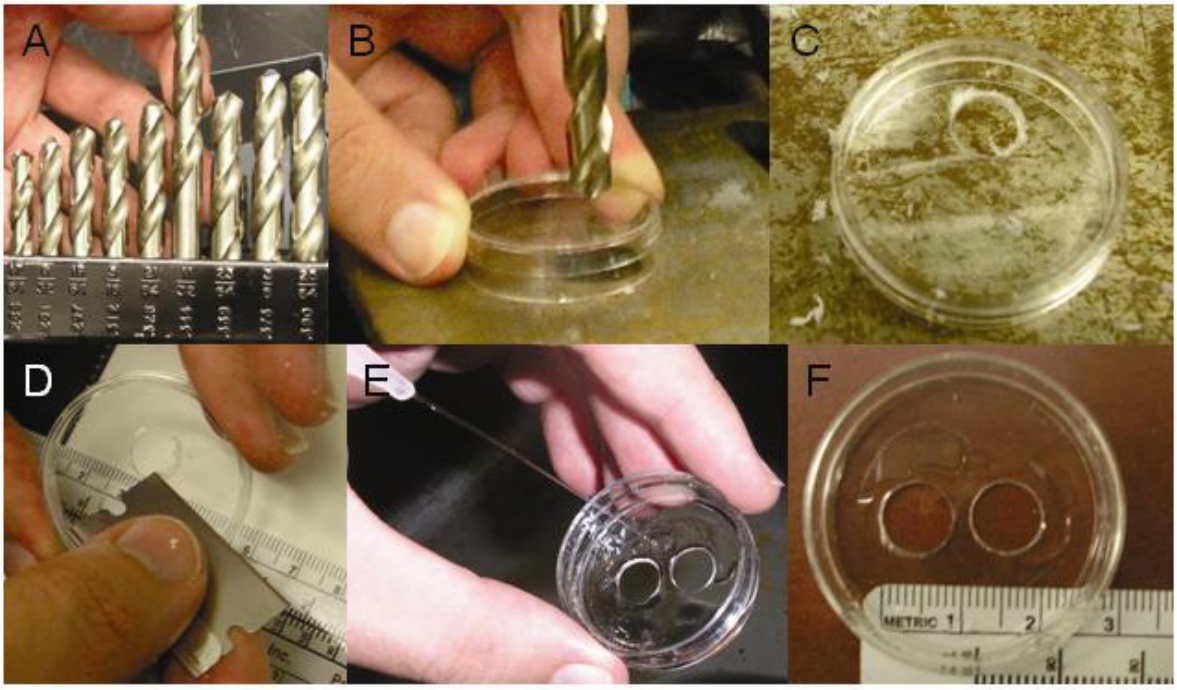
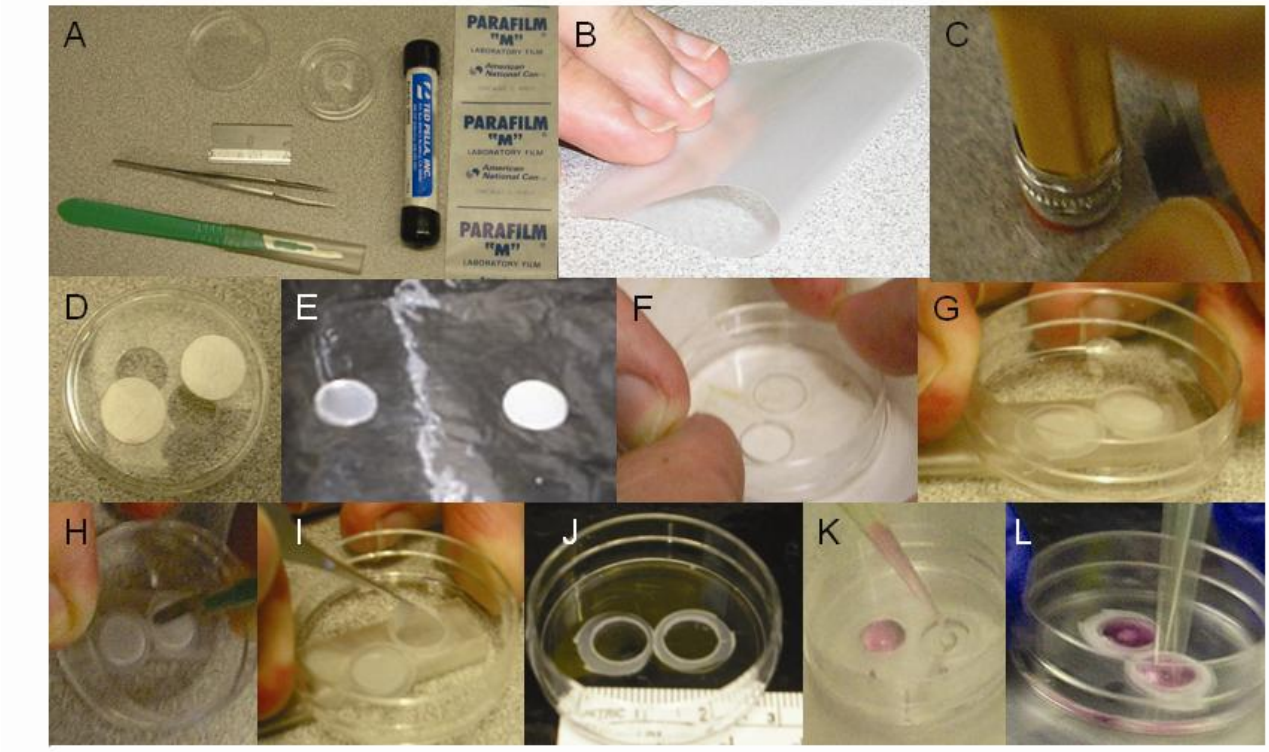
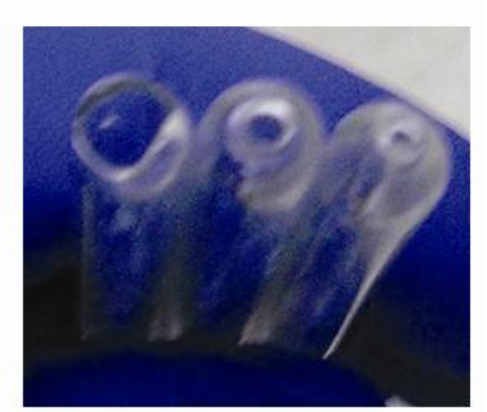
Supplemental Materials



Supplemental Figure 1: Drilling Wells. An $^{11}/_{32}$" bit (A) was used to drill the bottom face of a 35mm Falcon Petri dish (B). This configuration results in the plastic burr on the outside of the dish (C) where it can easily be removed (D). Following burr removal dishes are washed in 70% ethanol (E). If increased plating area is desired, extra wells may be drilled (F) rather than increasing the diameter.



Supplemental Figure 2: Sealing Wells and Laminin Coating. Materials (A). Parafilm is folded to double thickness (B) and then cut with razor using the coverglass as a template. A pencil eraser serves to immobilize the coverglass during cutting (C). Finished Parafilm discs (D) are placed over coverglass on a hotplate at a low setting. When ready, Parafilm transitions from opaque (E, right) to transparent (E, left), but should not become liquid. The previously drilled Petri dish is pressed over the Parafilm (F) to seal the well and then allowed to cool. A scalpel cover (G) is used to support the coverglass while the Parafilm inside the well is cut (H) and removed (I). The finished dish (J) should be tested with water or buffer for several hours prior to use. 6µl laminin stock (at 1mg/ml) is plated cold onto bare glass in each well (clear droplet, K). Cold MEM is used to cover laminin (K) and the glass is scratched gently with a pipette tip to ensure adequate diffusion to the entire surface (L). Dishes are allowed to sit for at least one hour prior to removal of laminin solution and rinsing with MEM.



Supplemental Figure 3: Trituration Pipettes. From left to right: Large bore, useful for samples containing pieces of bone; medium bore, useful for fragile samples free of bone (soleus, deboned intercostal); small bore, used only for short muscle fibers/small cells (FDB, non muscle tissue).

Supplemental Video 1: Intercostal Muscle Fiber. Single twitch followed by 10Hz train in response to field stimuli (1ms, 18v). Timescale = 33ms/frame.

Supplemental Video 2: FDB Muscle Fiber. Single twitch followed by 10Hz train in response to field stimuli (1ms, 18v). Timescale = 33ms/frame.

Supplemental Video 3: Soleus Muscle Fiber. Single twitch followed by 10Hz train in response to field stimuli (1ms, 18v). Timescale = 33ms/frame.