Protocol graciously provided by: Dr. Mariano Viapiano, Viapiano Lab protocols (© 2006-2014)

M. Cell Recovery from nanofibers

1. To passage cells from nanofiber scaffolds to other cultureware the cells can be treated with trypsin, accutase, or other appropriate enzyme as if they were on conventional cultureware. Avoid using cell scrapers. Instead, gently dislodge the cells by careful pipetting and tapping on the culture plates
2. To prepare cell lysates the cells can be treated with the appropriate lysis buffer directly on the wells. Aqueous buffers without chaotropic agents (urea, guanidine HCl) will not affect the fibers.
3. Lyse the cells for 20 min on ice and scrape the nanofibers with a pipette tip to prepare a total lysate in the well. Collect the lysate with a pipette and transfer to a suitable tube
4. Centrifuge the tube for 10 min at 10,000*g* to remove insoluble fibers and cell debris
5. To extract RNA/DNA, cells can be collected in tubes as indicated above or the RNA extraction solution (eg, Trizol or similar) can be added directly to the wells and recovered by pipetting (proceed as if using conventional cultureware). This will melt and dissolve the fibers, therefore, the cell/fiber suspension must be centrifuged (10 min at 10,000*g*) to remove insoluble debris before continuing with RNA/DNA extraction