Isolation of primary mammary organoids
From: A.J. Ewald, A. Brenot, M. Duong, B. S. Chan and Z. Werb, Collective Epithelial Migration and Cell Rearrangements Drive Mammary Branching Morphogenesis, Dev. Cell 14 (2008) pp. 570-581

Cultures were prepared as previously described (Fata et al., 2007). Briefly, glands were minced and tissue was shaken for 30 min at 37°C in a 50 ml collagenase/trypsin solution in DMEM/F12 (GIBCO-BRL), 0.1 g trypsin (GIBCO-BRL), 0.1 g collagenase (Sigma C5138), 5 ml fetal calf serum, 250 μl of 1 μg/ml insulin, and 50 μl of 50 μg/ml gentamicin (all University of California, San Francisco Cell Culture Facility). The collagenase solution was centrifuged at 1500 rpm for 10 min, dispersed through 10 ml DMEM/F12, centrifuged at 1500 rpm for 10 min, and then resuspended in 4 ml DMEM/F12 + 40 μl DNase (2U/μl) (Sigma). The DNase solution was shaken by hand for 2–5 min, then centrifuged at 1500 rpm for 10 min. Organoids were separated from single cells through four differential centrifugations (pulse to 1500 rpm in 10 ml DMEM/F12). The final pellet was resuspended in the desired amount of Growth Factor Reduced Matrigel (BD Biosciences).

Fata et al., 2007 J.E. Fata, H. Mori, A.J. Ewald, H. Zhang, E. Yao, Z. Werb and M.J. Bissell, The MAPK(ERK-1,2) pathway integrates distinct and antagonistic signals from TGFα and FGF7 in morphogenesis of mouse mammary epithelium, Dev. Biol. 306 (2007), pp. 193–207.