The inability of the heart to regenerate damaged tissue is a main barrier for the recovery of cardiac function. Studies using human induced pluripotent stem cells (hiPSCs) have shown great potential to replace damaged cardiomyocytes post-cardiac infarction. Several methods have been developed for increasing cardiac cell yields, however, optimization of cardiac differentiation rates and potency is still needed for clinical trials and standardization of cell manufacture. Cell organization and sarcomere orientation have been shown to correlate with improved cardiac function. We hypothesized that fibrous substrates of defined orientation will enhance cardiac cell organization to increase cardiac cell yields and potency of hiPSCs-derived cardiomyocytes. To test our hypothesis, electrospun fibrous substrates of defined orientation composed of Collagen type I or Polycaprolactone (PCL) were developed and characterized for cell culture studies. Collagen type I was selected as a natural polymer which is abundant in the extracellular matrix of the adult myocardium. Also, PCL was selected as a synthetic biocompatible polymer due to the resistance to cell degradation and its commercial availability. Collagen substrates were synthetized using a solution of collagen type I sponges and acetic acid through the electrospinning technique. Matrix porosity, orientation, and fibril diameter were quantified from Scanning Electron Microscopy (SEM) micrographs using ImageJ software and stiffness from Instron Machine. Substrates of similar physical properties were grouped as random or aligned based on the level of fibril orientation. Substrates were bound to double–sided medical grade tape to fix the substrate to the bottom of the culture plates prior to cell seeding. For cardiomyocyte differentiation, WTC-11 and H9 cells were seeded on tissue culture plastic matrigel-coated wells (TCP/MG) and fibrous substrates using small-molecule inhibitors of Wnt signaling (GsK3 inhibitor and Wnt inhibitor) as previously described in Lian et al. Expression of pluripotency (OCT-4 and TRA-1-60) and cardiac cell markers (cTNT and cTnI) was monitored over 15-30 days to study maturation characteristics. Flow cytometry analysis of the cells on collagen substrates showed that random but not aligned fibrils reduced the expression of pluripotent markers by ~20% in the H9 cell population suggesting a loss of pluripotent capacity. The percentage of cardiomyocytes was improved on collagen fibrous substrates, but no significant difference was found between random and aligned in the H9 cell line. Cell orientation across the different substrates in H9 cells showed an increase on the elongation factors during the analysis of nucleus alignment. The percentage of cardiomyocytes using WTC-11 cells was significantly improved by 20-30% on collagen fibrous substrates with fibronectin coating as compared to PCL or TCP/MG. Electrospun collagen type I fibrous substrates are a potential alternative to Matrigel coated surfaces to guide cardiac differentiation of hiPSCs.
Figure 1. Cardyomiocytes differentiation on H9 cells (Left graphic) and WTC-11 cells (Right graphic) on different substrates: tissue culture plastic with a coating of matrigel (TCP/MG), random collagen fibril (RCF), RCF with Fibronectin coating (RCF/Fib), aligned collagen fibril (ACF), ACF with Fibronectin coating (ACF/Fib) and polycaprolactone (PCL). Data represent the average mean +/- SE of 3 independent experiments with n=3.