Sphingosine-1-phosphate (SIP) is a biologically active lipid mediator with a lot of pivotal roles in the regulations of cell growth, migration, differentiation and apoptosis. However, the signal transduction promoted by SIP in human dermal fibroblasts is still unclear. We investigated the signal transduction by SIP in human fibroblasts using collagen matrices contraction. This study aims at investigating whether or not SIP has the possibility to apply the treatment for cutaneous wound healing in the future. We found that SIP promoted floating collagen matrices contraction (FMC) as a model of initial phase of wound contraction, and some kinds of G protein, Giα, Rac1, Rho and ROCK (Rho associated coiled-coil forming kinase) were involved in SIP promoting FMC. However, ROCK was considered to be partially involved in SIP promoting FMC. mDia as well as ROCK have been recognized to be putative downstream target molecules of Rho. In mDia-silenced cells, ROCK inhibitor suppressed the stress fiber formation regardless of the presence or absence of SIP. Our results indicate mDia as well as ROCK may be involved in the downstream of Giα, Rac1 and RhoA on developing actin stress fiber of human dermal fibroblasts with SIP stimulation.