Recently the growing need for quick and reversible attachment in different fields of engineering and technology has led to the development of the new emerging field of adhesion science. This has been essentially inspired by those animals and insects that during their natural evolution have developed fantastic biological attachment systems allowing them to adhere to and run on walls and ceilings of uneven surfaces comprised of different materials and various contact conditions. Examples of potential applications of engineering biomimetic adhesive microstructures, include climbing robots, handling systems for wafers in nanofabrication facilities and mobile sensor platforms. So far, the adhesion and friction abilities of such biomimetic adhesive microstructures have been usually evaluated against hard and smooth counter-faces (substrates), often glass. However, possible future non-conventional uses of these biomimetic adhesive surfaces, such as in biomedical field, require adaptation and optimization of their tribological properties (friction, adhesion and peeling) in contact with soft and rough substrates that can simulate biological tissue properties. This study investigates the influence of the mechanical proprieties, surface roughness, and contact environment conditions on the adhesion ability of biomimetic adhesive microstructure in form of micro-mushrooms. Biomimetic adhesive micro-mushroom shaped microstructures were fabricated by a casting process from polyvinyl siloxane (PVS), while the substrates (counter-faces) were prepared by replicating various surface roughness of different objects. The rough counter-faces were prepared with three materials; (i) soft, made of PVS (poly-vinyl siloxane), (ii) soft, made silicone rubber impression material (SILFLO), and (iii) hard, made of epoxy and used as a benchmark. The topography of the different samples made of the three materials was fully characterized using SEM and 3D optical profilometer, and the various roughness parameters were measured. The adhesion achievements of the proposed engineering biomimetic adhesive microstructures, when contacting the different counterfaces (different materials and different roughness, were evaluated under different contact and loading conditions (preload, velocity, and environment) using a customized two-axis test-rig specially designed for this purpose. Results show that preload has a very limited influence on the generated adhesion force. Under dry contact condition, hard counter-face leads to a higher adhesion force compared to a soft counter-face. This behavior can be related to difference interfacial stress distributions. Under wet contact condition, soft counter-face materials seem to generate better adhesion that hard ones. This enhancement can be explained by an additional adhesion force by capillarity.