Commercially available prostetic heart valves (biological and mechanical) are used for the surgical treatment of heart valve defects, however they still have some limitations. Therefore, the search for prostheses with improved hemodynamics and biomechanical properties possible for cardiac surgery is ongoing. Tissue engineering offers promising alternatives to the existing solutions on the market. One strategy is the modification of xenogeneic tissues, this would limit the immunogenicity of the implant, and at the same time would enable the natural processes of bioprosthesis remodeling. This concept was explored in this this work. Biological scaffolds obtained by decellularization of the porcine pulmonary heart valve were used for the study. Two methods were used to remove the cells - enzymatic (D1) and detergent (D2). Additionally, in order to cross-link and stabilize the structures of extracellular matrix (ECM) of scaffolds, low concentrations of glutaraldehyde (GA - 0.0125%, 0.025%, 0.05%) were tested. The aim of the study was to compare both methods D1 and D2 and to examine the effect of applied GA concentrations on the biomechanical and biological properties of the obtained matrices, which were compared to the native tissue (N). Scaffolds were examined for the degree of cell removal from tissue, the effect of decellularization on ECM structures, scaffold susceptibility to enzymatic degradation and calcification, as well as biomechanical properties. The cytotoxicity of the obtained matrices was also checked, as well as the possibility of colonization by endothelial cells and the response of these cells to the contact with the modified ECM. The studies presented that the applied decellularization methods - D1 and D2 differed in the efficiency of removing cells from the tissue in favor of the D2 method, which showed an additional visible advantage in the preservation of ECM structures. Both methods significantly influenced the biomechanical properties of the tissue and the use of low concentrations of glutaraldehyde gave satisfactory results only in the case of scaffolds obtained by the D2 method when treated with extreme concentrations of GA (0.025% and 0.05%). The degree of the calcification of scaffolds was different for the examined fragments of the pulmonary valve - vessels (n) and leaflets (p) and depending on the used GA concentration. Only the scaffolding of the leaflets obtained by the D2 method showed no signs of calcification. The use of selected concentrations of glutaraldehyde enabled the colonization of modified scaffolds with endothelial cells, however, it was noted that the increase in the cytotoxicity effect was correlated with the method of obtaining scaffolds - D1. The above observations allow the conclusion that the use of glutaraldehyde in concentrations of 0.025% and 0.05% in combination with the tissue decellularization method - D2, allows optimization of biomechanical properties of collagen scaffolds while reducing the cytotoxic effect.