

**NATURAL POLYMER-BASED MATERIALS FOR IN VITRO 3D SKIN MODEL
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SUMMARY

Nowadays, a three-dimensional (3D) in vitro skin model has been widely studied and used to analyze the various type of drugs effect. Collagen is highly recommended natural material in developing artificial 3D bioscaffold to mimic the extracellular matrix organisation of native skin. This study aimed to develop the microporous structure of collagen sponge via freeze-dry technique, evaluate the cellular compatibility, and its ability to form epidermal- and dermal-like skin structure using co-culture skin cells. In brief, the collagen type I (col-I) was extracted and purified via acid-based extraction method followed by the dialysis process. The col-I solution was poured into the desired mould and pre-frozen at -80 °C for 6 hours followed by the freeze-drying. The col-I sponge was post-crosslinked with genipin (GP) and carbodiimide (EDAC) as a natural and synthetic crosslinking agent, respectively. The gross appearances and collagen content visualisation were performed by using picosirius red dye. The cellular toxicity and growth profile were analysed via live and dead assay, and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, respectively. Micrograph evaluation of cell morphological feature and cell migration was captured by scanning electron microscopy at two different microenvironments of the submerged and air-liquid interface (ALI). The col-I sponge crosslinked with GP visualised light brown appearance as compared to other treatment groups. All col-I sponges presented dark red in color that demonstrated the presence of collagen type I composition. The EDAC-crosslinked col-I sponge was identified toxic to the co-culture skin cells than that of other treatment groups. The cell attachment and proliferation was low in the EDAC-crosslinked col-I sponge as compared to others. The micrograph evaluation revealed that both skin cells could migrate and form two different skin layers containing epidermal- and dermal-like structure at both culture conditions among treatment groups. However, both cell attached to EDAC-crosslinked col-I sponge demonstrated the morphological features of the apoptotic cell. In conclusion, these results provide a valuable information of 3D in vitro skin model development to test cosmetic products and drugs, by using co-culture skin cells on col-I bioscaffold with a various crosslinking agent at different physiologically realistic microenvironments.