

To satisfy the increasing medical demanding especially for sever burn patients to regenerate full thickness wound cure, this study developed dermis with gelatin based scaffold and perform the biocompatibility tests. To prepare scaffold 30% of gelatin was mixed with sieved salt and dried in the mold to shape then, cross linked with a water-soluble cross-linker, EDAC. Preparing the cell for seeding from a rabbit skin, the fibroblast and keratinocyte were successfully isolated and cultured in vitro. After cell and scaffold were ready, the fibroblast was seeded to the scaffold ( $\sim 10^6$  cell/cm<sup>2</sup>) for preparing dermis and keratinocyte was cultured until forming the sheet. As a result for identity test for cells the morphology in the inverted microscope and histochemical staining were used and typical shape of each cell were shown. As the characteristics of scaffold the water uptake rate was  $18,900 \pm 800\%$  and the SEM image showed the porosity  $70.09 \pm 0.9\%$  and the bio-safety test include sterility showed no contamination. The growth rate of fibroblast was checked from passage 0 to 3 and the result showed very similar pattern of growth at each passage. The histochemical result of seeded cell's attachment, proliferation and distribution was confirmed from day 1 through 15. As the biocompatibility tests for the scaffold and cell seeded one, in vitro cell cytotoxicity with various cells include L929, skin irritation test, intracutaneous reactivity test and sensitization test were performed and no significant reaction was demonstrated. With the in vitro cultured keratinocyte sheet and dermis we developed, the attachment agent fibrin will be adjusted and full thickness artificial skin will tested with transplantation. As far the salt leaching method is very useful in developing artificial skin and gelatin scaffold is very promising material for the skin cell attachment and growth and both scaffold and artificial dermis is biocompatible and bio-safe.

[PA2-5] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Honokiol induces apoptosis in activated rat hepatic stellate cells via cytochrome c release and caspase activation**

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The therapeutic goal in liver fibrosis is to reverse fibrosis and selective clearance of activated hepatic stellate cells (HSCs), which play a central role in liver fibrogenesis, by apoptosis might be essential during resolution of fibrosis. Past several years we screened for natural products which mediate apoptosis in activated HSCs, and among the candidates honokiol, isolated from Magnoliae Cortex, was found to induce apoptotic death in activated rat HSCs in a dose- and time-dependent manner at the concentration between 12.5 microM and 50 microM. Apoptosis was determined by detection of DNA fragmentation in gel electrophoresis, morphological alternations by flow cytometry and quantification of phosphatidylserine externalization by Annexin V labeling. Activation of caspase-3 and -9, and the proteolytic cleavage of poly(ADP-ribose) polymerase were found during apoptosis induced by honokiol. In addition, pan-caspase, selective caspase-3 and selective caspase-9 inhibitors but not selective caspase-8 inhibitor blocked honokiol-induced apoptosis. Honokiol induced the reduction of mitochondrial transmembrane potential and the release of cytochrome c into cytoplasm. And honokiol also down-regulated bcl-2 protein. Take together, our findings indicate that honokiol mediates apoptosis in activated rat HSCs through mitochondria alternations to caspase-9 and that then the downstream effector caspases are activated sequentially.

[PA2-6] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Baicalein induced Apoptosis of Rat Hepatic Stellate Cells**

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Baicalein (5,6,7-trihydroxyflavone), a flavonoid originated from the root of Chinese medicinal herb *Scutellaria baicalensis*, has been shown to exert anti-inflammatory and antioxidant effects and hepatic stellate cells play an important