

G: Summary

Perfusion chamber systems for development of organotypic co-cultures of fibrocytes and keratinocytes isolated from the bovine hoof

An organotypic dermoepidermal *in vitro* model of fibrocytes and keratinocytes isolated from the bovine hoof was developed basing on the investigations of NEBEL (2005). The model was characterised and challenged experimentally. Enzymatic methods for the isolation of cells were adapted to the specific properties of claw tissue in order to optimise the culture. Using these methods it was possible to separate dermis and epidermis precise and reproducible to obtain highly pure cultures of both cell types. The isolated primary cultures were characterised by means of morphological and immunohistochemical techniques.

Commercial available perfusion chamber systems were modified in a way that is was possible to use them to culture the isolated hoof cells. As the commercial chambers were suboptimal, novel chambers were developed and used for long term perfusion culture of the cells. Different to static cultures it was shown that both cell types proliferated faster, subsequently differentiated in an organ specific way and stayed vital over months. Filter materials were identified and tested experimentally, which were suitable as bio membranes in the chambers. These membranes provided the basis for the organotypic growth of cells in the chamber.

With the optimised perfusion chamber systems fibroblasts and keratinocytes deriving from claw tissue were cultured three dimensionally. Light and electron microscopic as well as immunohistochemical examination proof that the spatial arrangement, i.e. the tissue architecture and the differentiation of cells *in vitro* largely resembled the situation *in vivo*. The culture in perfusion chambers successfully simulated an *in vivo* situation *in vitro*. These organotypic cultures were used for a series of challenge experiments to examine the effects of selected growth factors and cytokines, which are believed to play a role during the pathogenesis of bovine laminitis. The effects of four factors (*Keratinocyte growth factor*, *granulocyte macrophage-colony stimulating factor*, *interleukin 1-alpha* and *tumor necrosis factor-alpha*) on cellular proliferation and differentiation were examined *in vitro*. The results provide evidence that the proliferation of the horn producing keratinocytes is modulated by these factors. Therefore these are promising candidates for continuative examinations on the physiopathology of bovine laminitis.

The novel organotypic cultures and perfusion chambers developed and tested in the work presented here are powerful tools for future research on dermoepidermal interactions during

the pathogenesis of bovine laminitis and on the mechanisms involved. Beyond this, the systems and the associated technology provide a basis for studying related problems in other species, e.g. laminitis in horse and its pathomechanisms. Furthermore, the systems presented here could provide the basis for investigation of the regulatory mechanisms in other tissues and organs, such as skin or placenta, where dermoepidermal regulation plays a role in maintenance of tissue integrity and development of disease.