

Poster: strategies to reduce animal numbers for testing biologicals

The hen`s egg testing on the chorioallantoic membrane – a model for multiple purposes

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The chorioallantoic membrane of the fertilized and incubated chicken egg emerges around embryonic day 6, fusion of chorion and allantois are forming a membrane, which primarily serves as a primitive respiratory system for the chick embryo, releasing CO₂ and absorbing O₂. Due to its function as respiratory organ the chorioallantoic membrane is highly vascularised, providing a network of fine capillaries in which gas exchange occurs. Tests conducted on the chorioallantoic membrane can be regarded as painless for the chick embryo for two reasons: the absence of neural cells in the chorioallantoic membrane itself and the fact that the neural system of the chick embryo has not been completely developed until embryonic day 11 (fusion of the neural tube).

Testing of the potential irritancy of different chemicals for the human eye is an established model of tests conducted on the chorioallantoic membrane and is one alternative to animal testing (Draize test). The tested substance is directly applied onto the chorioallantoic membrane and reactions, such as haemorrhage, intravasal coagulation or lysis of blood vessels, are investigated in course of time. In a slight modification of this test different materials as used for medical implants can be tested for their biocompatibility.

Another field of application using the chorioallantoic membrane is cancer research and the study of angiogenesis. We are testing the growth behaviour of different tumour cell lines on the chorioallantoic membrane in order to establish an alternate model to animal testing. Therefore the following protocol was developed: On embryonic day 5 a hole is drilled at the pointed pole of the egg shell and 3 ml albumen are removed by a syringe. The remaining hole is sealed with wax. At the following day the egg is carefully cracked at the blunt end and the shell membranes are removed with sterile forceps. A silicone ring is placed onto the chorioallantoic membrane in which 2 x 10⁶ tumour cells suspended in 20 µl medium are given. The egg is sealed with autoclaved aluminium foil and incubated at 37°C and 95% rh until day 11. Pictures are taken daily to document tumour growth. At day 11 the chorioallantoic membrane are fixed *in ovo* with 4% formalin and the tumour areal is excised, embedded in paraffin and investigated histologically and immunohistochemically.

Until now we tested different cell lines in this model:

- HoMel A1 and L1 (horse melanoma) and MelJuso (human melanoma) are unable to invase the chorioallantoic membrane, but remain swimming in the thin layer of albumen which covers the chorioallantoic membrane.
- 22RV1 cells (human prostate carcinoma) form solid tumours on the chorioallantoic membrane, but are unable to transcend the basal lamina and grow invasive.
- SaOs 2 cells (human bone sarcoma) form solid tumours and grow invasive, but very slowly, so only very few cells enter the chorioallantoic membrane.
- MCF 7 cells (human breast carcinoma) form solid tumours and grow invasive.

Furthermore SKBR3 (human breast carcinoma), Lovo, sw620 (both human colon carcinoma) cells are tested in this model system.

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