Culture of Differentiated Adult Rabbit Auricular Chondrocytes

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Chondrocytes dedifferentiate to a fibroblast-like phenotype on plastic surfaces. Dedifferentiation is reversible if these cells are then cultured embedded in gels as alginate, agarose or collagen. Chondrocytes cultured in suspension on a non-adherent surface are also known to form aggregates of differentiated cells. The knowledge of chondrocyte behavior in culture is relevant for tissue engineering purposes. In this report we describe a simple method to culture differentiated or redifferentiated rabbit auricular chondrocytes on plastic surfaces with a stable phenotype. When chondrocyte aggregates formed in suspension are next seeded on plastic surfaces, most of them attach to the plastic as round or polygonal cells, and this morphological differentiation, confirmed by the presence of type II collagen, is stable for long culture periods. We also report that the addition of aggregates to monolayer cultures of dedifferentiated chondrocytes results in their redifferentiation, as is shown by their morphological changes and the synthesis of type II collagen. Therefore, this simple method can be useful for the study of chondrocyte behavior on plastic surfaces and for redifferentiating previously proliferated chondrocytes in tissue engineering techniques. Furthermore, these results demonstrate that, in addition to culture conditions such as cell isolation method or cell-density, chondrocyte behavior on plastic depends on the presence or absence of aggregates resulting from the dissociation process.

Histamine and VIP-positive Mast Cells in Renal Blood Vessels of Domestic Swine

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Introduction: Histamine and vasoactive intestinal polypeptide (VIP) are well known biogenic amines (substances), which rapidly elute from the mast cell granules under physiological conditions. They play an important role in the smooth muscle cells motility.

Aim: The aim of the study was to investigate histamine- and VIP-positive mast cells in the wall of pig kidney blood vessels in concern to their role of motility of smooth muscle cells and renal haemodynamics, respectively.

Material and Methods: Extra- and intraorgan renal blood vessels taken immediately after slaughtering of eight 6–8 months pigs were studied. Tissue samples were immersion fixed in 0.05 M cacodylate buffer, pH 7.2, containing 2% paraformaldehyde and 0.2% glutaraldehyde for 5 days at 4°C. After fixation, tissue blocks were rinsed in 30% sucrose in distilled water at 4°C for 1–3 days. Cryostat sections (20–30 μm thick) were prepared and thawed in 0.05 M cacodylate buffer, pH 2, overnight. Immunocytochemical procedure started for free-floating sections which were incubated in 1.2% hydrogen peroxide in methanol for 30 min, and rinsed in 0.1 M phosphate buffered saline (PBS), pH 7.4, for 15 min. Afterwards, sections were incubated with the primary antibodies in a dilution of 1:100 (histamine) and in vasoactive intestinal polypeptide (VIP) 1:1000 for 24 h at room temperature (RT), then rinsed in 0.1 M PBS, pH 7.4. Incubation succeeded: first, with biotinylated anti-rabbit (histamine) and anti-mouse VIP antibodies in a dilution of 1:20 for 4 h, and rinses in 0.1 M PBS, pH 7.4, and in 0.05 M tris-HCl buffer, pH 7.5, for 10 min. Peroxidase activity was localized by using a mixture of 3 mg 3,3' diaminobenzidine (DAB), in 15 ml 0.005 M Tris-HCl buffer, pH 7.5, and 36 ml 1 % hydrogen peroxide for 10–20 min. Finally, a rinse in 0.01 M PBS, pH 7.4, was undertaken.

Immunochemicals: The antibodies used were rabbit anti-histamine (H7403) and rabbit anti-vasoactive intestinal peptide (VIP), or (PEPA41) (Sigma, Saint Louis, Missouri, USA).

Results and Discussion: It was established that VIP-positive mast cells were located in the media and in the adventitia of large arterial vessels and renal vein, as well. Histamine positive mast cells were found mostly in the intima of renal artery and in the other layers of the wall. Both VIP and histamine mast cells were observed next to small arteries and veins in the fat tissue, around extraorgan blood vessels. Our finding suggest to mast cells participation in the smooth muscle cells motility and the maintenance of local homeostasis, as well.

Characteristics of Claw Horn Microstructure and the Effect of Flooring Systems on Horn Quality

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Introduction: Interactions between horn quality and flooring systems significantly impact on claw health. Poor horn