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Potential use of fish collagen matrix to evaluate tubulogenesis *in vitro*

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Formation or sprouting of new blood vessels (angiogenesis) is a complex process that involves the extracellular matrix (ECM) and endothelial cells (EC). A common *in vitro* angiogenesis assay consists of culturing EC on or inside distinct ECM components such as collagen. Recently, collagen from aquatic sources has gained attention in tissue engineering applications. Thus, in this work the potential of fish collagen from *Pygocentrus nattereri* (CP) to induce tubular structures in tEnd.1 (RRID: CVCL_6272) cell line was evaluated. Firstly, collagen from discarded fish skin was extracted by acid solubilization, concentrated by salt precipitation, dialyzed against 100 mM acid acetic and preserve at 8°C. The hydroxyproline analysis revealed a collagen concentration of 6,9 mg/mL (73,8 % of total protein content) and the electrophoretic pattern (SDS-PAGE under non-reducing conditions) showed that extracted CP was type I. To evaluate CP use in angiogenesis assay, 100 µL of a 0.5 mg/mL solution (culture medium-pH 7) was added to each well of 96-well plate and incubated for 30 min at 37°C. Endothelial cells (30.000 cells/well DMEM-5%FBS) were seeded on collagen-coated wells and incubated for 24h at 37°C-5%-CO_2. Controls were performed using untreated wells. Tube formation was monitored by an inverted phase contrast microscope and images were taken with a digital camera. Using ImageJ software, a number of capillary structures was analyzed. EC cultured in collagen matrixes organized rapidly into an irregular network, which was formed by many tubular structures radiating out from cell aggregates. These results suggest the potential use of fish collagen in cell culture.