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RECOMBINANT CLASS-I AND CLASS-II COLLAGENASES: NEW FORMULATIONS IN CELLS EXTRACTION

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The major component of extracellular matrix is the collagen and collagenase enzymes are used to extract cells from biological tissues, which the challenging goal is to obtain a high number of healthy and living cells. The current collagenases utilized for regenerative medicine and cell therapy are extracted from the culture of *Clostridium Histolyticum* and the subsequent purification thereof of the bacterial proteins produced. The result of this process leads to a blend containing different percentage ratios of two main collagenase isoforms (class I and class II) plus a number of other lytic enzymes (clostripain, trypsin like, caseinase activity, etc.). Such blends present many limitations in terms of lot-to-lot consistency, variable enzymatic activity and purity. In order to better control the extraction processes and the enzymes formulation, we have generated recombinant collagenases of class-I (COL G) and class-II (COL H), which allows efficient, customized and standardized cell extraction procedures. These recombinant enzymes were used together with thermolysin (as a generic proteolytic enzyme) in the extraction processes of different cell types, for which the quantities of the two classes of collagenases plus the neutral protease were precisely defined. The current extraction procedure, with collagenases from *C. histolyticum*, is based on a formulation that use a weight collagenase ratio, due to the impossibility in determines the exact enzymes activity for each class of collagenases. Our study performed with COL G and COL H to extract *Langerhans* islets from rat pancreas highlighted how this formulation lead to variable results, while the formulation based on the enzymatic activity ratio (COL G : COL H, determined with the Grassmann method) allows a standardized and reproducible cell extraction. Based on this results, several extraction protocols have been improved, such as: cardiomyocytes from rat heart, chondrocytes from nose or cow's hoof cartilage, hepatocytes from rat liver, osteoblasts from rat skull cap and mesenchymal stem cells from rat adipose tissue. Each protocol was optimized, using as parameters the phenotype and the number of extracted cells, but also performing functional and /or differentiation assays.

Biography

Giulio Ghersi is Professor in Biochemistry and Applied Biochemistry in Biotechnology, Element of Biochemistry and Cellular Biology in Medical Engineering. Vice-Director Advanced Technology Network Center (ATeN Center) University of Palermo.CEO of ABIEL s.r.l. (www.abielbiotech.com) a spin-off of the University of Palermo and of the Council National of Research (C.N.R.) IAMC.PI of Mediterranean Center for Human Health Advanced Biotechnology (PONa3_00273 23 M€)PI of "SIB: Advanced solutions using biomaterial by composite matrix in repair and regeneration of articular cartilage using non invasive techniques (PON01_01287 1,6 M€).Unit ABIEL PI for Horizon 2020 project "Diabetes Reversing Implants with enhanced Viability and long-term Efficiency – DRIVE" (0,9 M€).The research activities of greatest interest are currently directed to the optimization of the extraction processes, of cells for applications in the field of regenerative medicine and tissue engineering, through the use of specific proteolytic enzymes. As well as their use in nanostructured systems for greater penetration into solid tumor masses, and the controlled release of drugs and / or biomolecules with antitumor activity.- Salamone M. et al (2016) "Proteolytic enzymes clustered in specialized plasma-membrane domains drive endothelia cell migration". PLOS ONE, vol.11. Dispenza C. et al.(2012) Minimal in Radiation Synthesis of Biomedical Functional Nanogels. Biomacromolecules, vol 13, p.1805-1817.

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