Extraction and characterization of sheep (*Ovis aries*) skeletal muscle extracellular matrix

Girish Patil Shivanagowda, Santhosh Kacham, Praneetha D C, Raja Balamanikandan, Ramakrishna C

ICAR - National Research Centre on Meat, India

Objectives: The Extracellular Matrix (ECM) is the noncellular component present within all the tissues and organs of the living or- ganisms. ECM provides physical scaffolding for the cellular constituents and also initiates essential biochemical and biomechani- cal cues which are required for tissue morphogenesis, differentiation and homeostasis. To develop the scaffolds for cultured meat production, understanding the structure and composition of the ECM is imperative. Reports on extraction and characterization of the Sheep (*Ovis aries*) Skeletal Muscle Extracellular Matrix (SSM ECM) are scarce. This work was undertaken to fill this gap.

Materials and Methods: In this study, sheep skeletal muscle was decellularized by two different methods: Sodium Dodecyl Sulfate (SDS) (Fernández-Pérez & Ahearne, 2019) and Trypsin (Rahman et al., 2018) based methods. Extracted SSM ECM samples were dried, frozen using liquid nitrogen and crushed into a coarse powder by motor and pestle. The dried extracellular matrix powder was treated with DNase (1mg/ml) for 12 h at 4 ºC for degrading the DNA. The DNA was isolated from 50 mg SSM ECM samples using the commercial tissue DNA isolation kit. Collagen content in the ECM was determined by estimating the hydroxyproline content (Ignat'eva et al., 2007). Scanning Electron Microscope (SEM) (Model: JOEL-JSM 5600) was used for imaging of the ECM. Myoblasts isolation and differentiation was undertaken as per Lubna et al (2017). The expression of myogenic markers *viz.,* Myo-D, Myo-G, MEF2C and Pax-7 was checked according to the Wang et al (2020). The sheep serum was collected by slaughter- ing healthy sheep in the experimental abattoir of ICAR - National Research Centre on Meat, Hyderabad, India.

Results and Discussion: The SSM ECM extracted by both Sodium Dodecyl Sulfate (ECM-SDS) and Trypsin (ECM-T) based meth- ods showed white transparent appearance and the DNA content was less than 50 ng/mg. Degradation and reduction in the quantity of nuclear material in the ECM was confirmed by Hematoxylin and Eosin (H & E) staining and the porous structure was confirmed using SEM analysis. The proteomic analysis was undertaken using Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis which confirmed the presence of various ECM proteins. The ECM generated was biocompatible for culturing and differentiation of myoblasts when used as coating in the place of collagen. In addition, filter sterilized Sheep Serum (SS) collected from the abattoir was tried to replace Fetal Bovine Serum (FBS) in the cell culture medium. We plotted a growth curve for the myoblasts cultured under different conditions like ECM-SDS, ECM-T, and collagen-coated (CC) plates either with DMEM/F12 medium containing FBS or SS. The growth curve patterns of myoblasts cultured with the ECM-SDS and CC coating were similar when compared with ECM-T coating. The population doubling time of the of the myoblasts cultured on CC, ECM-SDS, and ECM-T coating in FBS was 20.57, 20.22, and 19.76 h; in SS was 26.29, 21.45, and 20.38 h respectively. The differentiation of the myoblasts in to myotubes was confirmed by changing to differentiation medium on Day 6. More myotubes formation was observed in ECM-SDS coated plates, as compared with ECM-T and collagen-coated plates. Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR) and the agarose gel electrophoresis image showed that the cells cultured on ECM and CC coated plates were positive for the myogenic markers Myo-D, Myo-G, MEF2C, and Pax-7. Myoblasts could be successfully cultured on collagen-coated, ECM-SDS and ECM- T coated plates.

Conclusions: The study showed that the extra cellular matrix extracted from the sheep skeletal muscle can successfully be used in the place of collagen for coating in culture flasks. Study also revealed that the filter sterilized sheep serum can be used in place of fetal bovine serum without affecting the growth and differntiation of the myoblasts. These two findings will help in brining down the cost of culturing of the sheep myoblasts.

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