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POTENTIAL BIOACTIVE PROPERTIES OF PULP DERIVED EXTRACELLULAR MATRIX HYDROGELS

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INTRODUCTION

The use of biological scaffolds in regenerative endodontics has gained much attention in recent years. The search for a new biomimetic scaffold that contains tissue-specific cell homing factors could lead to more predictable tissue regeneration (1).

The aim of this study was to prepare and perform preliminary characterization of pulp derived Extracellular Matrix (P-ECM) hydrogels for regenerative endodontic applications.

METHODOLOGY

Freshly extracted bovine molar teeth were used to prepare P-ECM; Bovine dental pulp tissues were harvested. Samples were decellularized using trypsin/ethylenediaminetetraacetic acid (EDTA) and treated with a nuclease solution DNase, then lyophilized for 24 hours. Decellularized tissues were histologically evaluated, and the residual DNA was quantified and compared to native tissues. This was followed by fabrication of extracellular matrix hydrogel scaffold and optimization of its concentration to be 3mg/ml (2). The prepared scaffolds were evaluated for their protein content as well as protein release at 0,1- and 3-days using BCA protein assay. 3-(4,5- dimethylthiazol-2-yl)-2-5-diphenyl-2H-tetrazolium bromide (MTT) cell viability assay was done using Rabbit dental pulp stem cells (rDPSCs). Passage 4 cells were cultured (in DMEM supplemented with 1% penicillin-streptomycin and 10% FBS) and plated in uncoated or ECM-coated (200µl/well) 24-well plated at seeding density of 30000 cells/well.

Figure (1): sample harvesting, decellularization and pulp ECM derived hydrogel preparation. (a,b)extirpated bovine pulp tissues cut into small segments and decellularized using trypsin/ethylenediaminetetraacetic acid (EDTA) and treated with a nuclease solution DNase; (c) pregel solution inserted in 24 well plate ;(d) gelated hydrogel with concentration 3.00 mg/ml hydrogel.

RESULTS AND DISCUSSION

Bovine pulp tissues were successfully decellularized. Histological evaluation showed absence of nuclei, preservation of architecture, collagen and glycosaminoglycans contents. DNA was found to be below the cut-off point (50 ng/mg tissue) using Nanodrop spectrophotometer (32±2.4 ng/mg tissue) for P-ECM compared to (350.7±7.4 ng/mg tissue) for native pulp . P-ECM hydrogel maintained viability of rDPSCs as compared to cells cultured under controlled conditions.

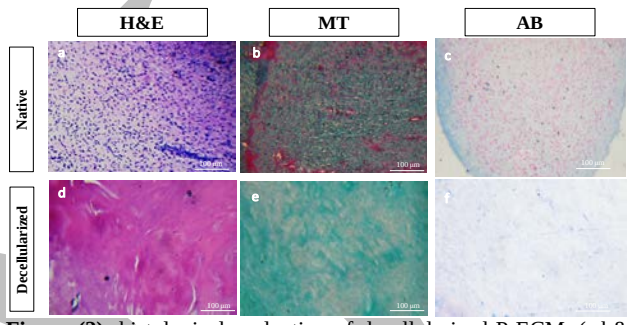

Figure (2): histological evaluation of decellularized P-ECM. (a,b&c) native pulp at 10x magnification stained with H&E, Masson's Trichome(MT) and Alician blue (AB) stains respictevly; (d,e&f) decellularized pulp showing absence of nuclei in all sections. Collagen bundles stained blue by MT stain. Gags content (stained faint blue by AB stain) is evident in both native and decellularized

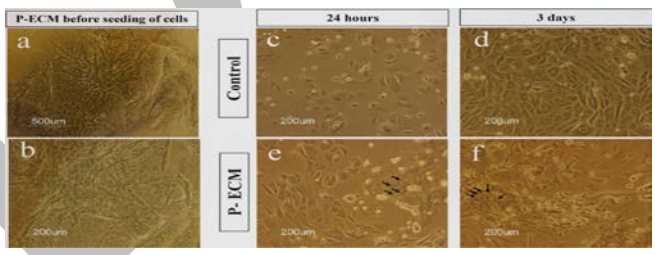

Figure (3): inverted phase contrast microscopic images. (a&b) showing the structure of P-ECM without cells at 4x and 10x respectively; (c&d) rDPSCs cultured under controlled conditions for 24 hours and 3 days respectively; (e&f) rDPSCs cultured on P-ECM for 24 hours and 3 days showing maintained viability and proliferation; black arrows in (e&f) pointing to P-ECM structure.

Total protein content was found to be 493.12 µg/µl. protein release was detected at time 0 showing burst release (109.6 µg/µl) followed by declined release at 24 hours (70.4 µg/µl) then increased release at 72 hours (102.7 µg/µl).

CONCLUSION

The pulp-derived extracellular matrix hydrogel scaffold retained its bioactive properties demonstrating a potential role as a scaffold for regenerative endodontic procedures.

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