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IN VITRO ASSESSMENT OF EMD BASED BIOMATERIAL BIOCOMPATIBILITY

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Abstract

Tissue engineering is a rapidly developing area that might restore, maintain or improve tissue functions. The major elements of tissue engineering are: integrate cells, scaffolds and biologically active molecules. These components act synergistically to regulate stem cell propagation and differentiation thus ensuring tissue regeneration. For evaluation of biocompatibility of enamel matrix derivative (EMD) based biomaterial, palatal mesenchymal stem (PMSCs) cells at a density of $1x10^5$ cells were co cultured. The level of adherence was daily assessed. The viability of cells was evaluated after 24h, 72h and 7 day using FDA (fluorescein diacetate) assay. Our study revealed that the selected biomaterial are biocompatible and can be used as scaffolds for mesenchymal stem cells delivery especially for periodontal regeneration.

Key words*: biomaterials, biocompatibility, stem cells, regenerative therapy.*

INTRODUCTION

Stem cells are definied as cells with clonogenic ability, self-renewing capacity that can differentiate in one or more specialized cell lineage (Rodriguez-Lozano et al., 2011). Biomaterial-based scaffolds are the most important tool in providing a 3D environment for cells, both in culture and inside the body. The main properties of biocompatible scaffolds (synthetic or natural) consist in optimal fluid transport, delivery of bioactive molecules, material degradation, cell-recognizable surface chemistries, mechanical integrity and the ability to induce signal transduction (Shin et al., 2003, Drury et al., 2003)

Natural biomaterials used for stem cells cultivation can consist of components found in the ECM such as alginate, cellulose, chitosan, collagen, fibrinogen, hyaluronic acid, silk fibroin, glycosaminoglycans (GAGs), hydroxyapatite (HA) etc., and therefore have the advantage of being bioactive, biocompatible, and with of similar mechanical properties as native tissue (Chung et al., 2008). Ideally the scaffold must provide certain properties (1) directed and controlled degradation; (2) promote cell viability, differentiation, and ECM production; (3) allow for the diffusion of nutrients and waste products; (4) adhere and integrate with the surrounding native cartilage; (5) span and assume the size of the defect, and (6) mechanical integrity depending on the defect location (Pati et al., 2012). Regenerative therapy requires such biomaterials and biocompatibility assessement of these cells is very important. Enamel matrix derivative, EMD (Emdogain®) (Straumann) is a purified acidic extract from the tooth germs of 6-month old piglets. The major component of Emdogain is a hydrophobic protein ameiogenin, is a widely used biologic agent

capable to support periodontal tissue regeneration (Koop et al., 2012, Gruber et al., 2013) formation of new cementum, periodontal ligament and alveolar bone (Miron et al., 2013).
The aim of the present study was to investigate the effects of EMD on palatal mesenchymal stem cells proliferation.

MATERIALS AND METHODS

Characterized palatal mesenchymal stem cells passages 7 (Roman et al., 2013) were used for our experiment. The cells were cultured in a 96-well plate in DMEM/F12 (Dulbecco's Modified Eagle's Medium/Nutrient F-12 Ham) (Sigma-Aldrich, St.Louise, MO, USA) supplemented with 10% FCS (fetal calf serum) (EuroClone, MI, Italy), 2 mM glutamine, 1% Non Essential Amino-Acids (NEAA) (Sigma-Aldrich, St.Louise, MO, USA). After 5 h of initial cultivation the culture medium were changed with serum free medium supplemented with EMD in different concentration (10, 20, 50, 100 µg/ml) for 24, 72h and 7 day. The level of adherence was daily assessed. The viability of cells was evaluated after 24h, 72h and 7 day using fluorescein diacetate (FDA) staining and the cells proliferation were evaluated using MTT assay. Experiments were performed in triplicate. All the results are expressed as mean ± standard deviation (SD). Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSIONS

Palatal mesenchymal stem cells used in our study were previously characterized for their stemness and trilineage differentiation capacity, performed according to the criteria of the International Society for Cellular Therapy (unpublished data). For assessments of EMD based biomaterials biocompatibility the cells were stimulated with four different concentration of Emdogain® (Straumann). Cells viability, proliferation capacity and the potential citotoxicity level were evaluated after 24h, 48h and 7 days. After EMD treatment the level of adherence were assessed daily.
After 24h of treatment the palatal mesenchymal stem cells were showed elongated phenotype a small part of the cells were in suspension, especially in culture treated with 20 µg/ml of EMD (fig.1, fig. 2).
The viability was assessed in three different periods, using MTT assay. The formazan were dissolved with dimethyl sulfoxide (DMSO), and the absorbance was measured at 550nm. The results were compared with control plates.

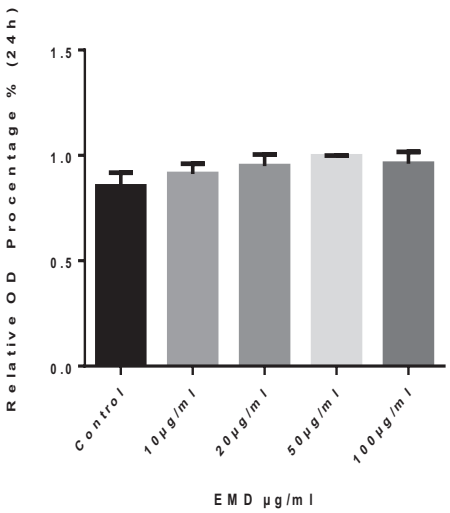
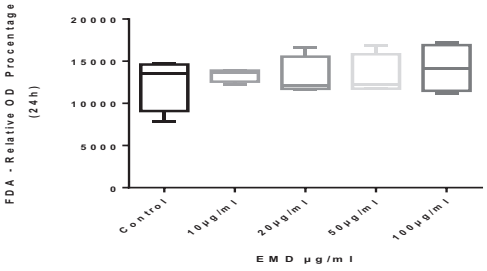


Figure 1. Palatal mesenchymal stem cells proliferation after suplimentation of propagation medium with EMD for 24h

FDA assay results reveal no significant difference compared with the control group.



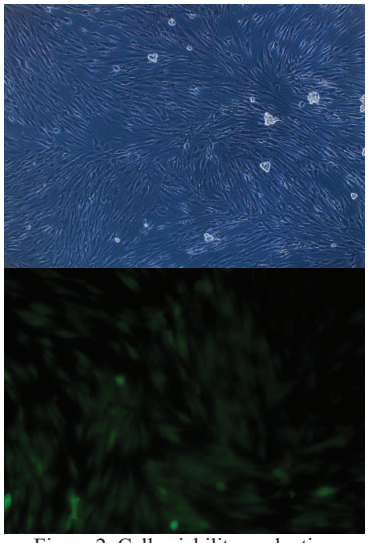


Figure 2. Cells viability evaluation using FDA assay

After 72 h of cultivation were observed an intense proliferation with a significant reduction of the non adherent cells (fig. 3, fig.4, fig. 5).

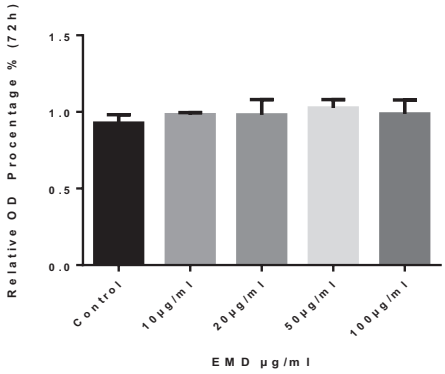


Figure 3. Palatal mesenchymal stem cells proliferation after suplimentation of propagation medium with EMD for 72 h

Also were observed organization of small cells clusters, in special in cultures treated with 50 and 100 µg/ml EMD. No citotoxicity were observed compared with control. Instead cell morphology shows some changes.

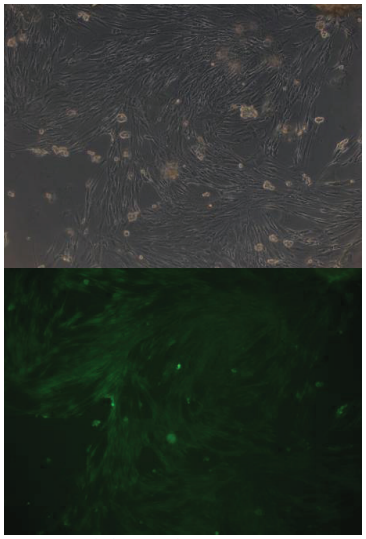


Figure 4. Viability evaluation using FDA assay

Compared with untreated cultures the degree of cell proliferation was significantly higher in cultures stimulated with EMD.

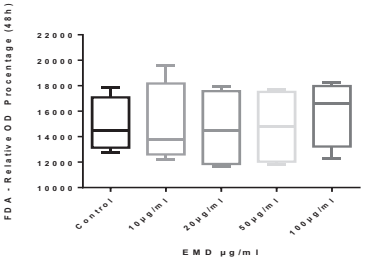


Figure 5. Cells viability after 48h of stimulation with EMD

There were no significant differences between the four concentrations (10, 20, 50, 100 µg/ml) of EMD in terms of toxicity.

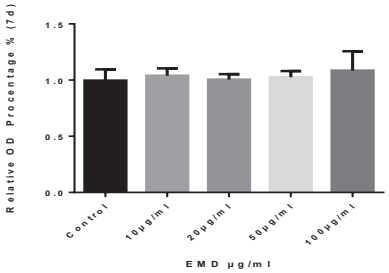
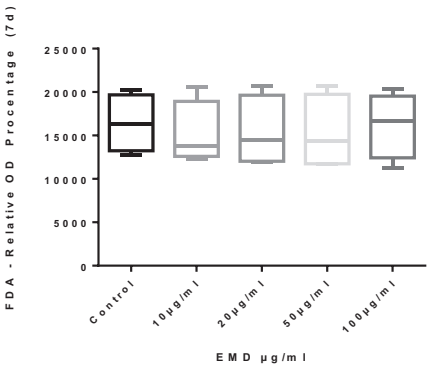


Figure 6. Palatal mesenchymal stem cells proliferation after suplimentation of propagation medium with EMD for 7 day

In 7 day in culture treated with 50 and 100µg/ml EMD the clusters increased in number and sizes (fig.6, fig.7).



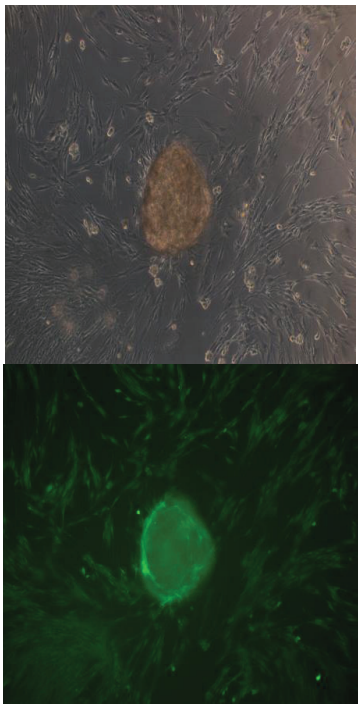


Figure 7. Cell cluster after 7 day of stimulation with EMD

In vitro studies indicated that EMD support cells proliferation and osteogenic differentiation (Gestrelius et al., 1997, Carinci et al., 2006, Wu et al., 2014). EMD also stimulate the signal transduction of bone morphogenic protein and transforming growth factor-ß and promote osteoclastogenesis (Hatakeyamaet al., 2006, Sculean et al., 2007, Fujishiro et al., 2008, Wu et al., 2014).

Mesenchymal stem cells (MSCs) which can be found in almost all postnatal organs and tissues (Patel et al., 2013) are stromal unspecialized cells that have the ability to self-renewal through cell division and also exhibit multilineage differentiation and immune-suppressive functions (Kim et al., 2012, Zhang et al., 2012, Patel et al., 2013).

Oral cavity such as dental pulp, dental follicle, dental papilla and periodontal ligament (PDL), coronal pulp, apical papilla, subepithelial layers of oral mucosa, the gingival tissues, exfoliated deciduous teeth have been identified as easily accessible sources of multipotent stem cells that could be cryopreserved and used for autogenic or allogenic cell therapy (Patel et al., 2013, Bojic et al., 2014).

An important subject for the development of differentiated cells is the practical aspects of producing optimal culture conditions (substrates, cultures medium, growth factors, etc) of these cells (Baharvand et al., 2005) for prolonged expansion (Ng et al., 2014) One of the major interests of regenerative therapy requires the cultivation of stem cells on different specific substrates namely degradable scaffolds to obtain all kinds of tissues through the control and guidance of their differentiation (Battista et al., 2005).

CONCLUSIONS

Our study revealed that the selected biomaterial are biocompatible and can be used as scaffolds for mesenchymal stem cells delivery especially for periodontal regeneration.

New biomaterials are being continuously developed, and their interaction with inserted cells and growth factors has a decisive role for regenerative medicine. Understanding the complex mechanisms involved in stem cells adhesion and division allows us to obtain useful biodegradable biomaterials combinations for cell therapy. Successful combination of these scaffolds lead to mimicking cellular microenvironment and maintaining pluripotent ability of these cells.

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