Journal of IMAB - Annual Proceeding (Scientific Papers) 2008, book 2

ISOLATION OF MESENCHYMAL STEM CELLS FROM THE PULP OF DECIDUOUS TEETH

Peneva M., Vanyo Mitev*, Nikolai Ishketiev*
Department of Children's Dental Medicine, Faculty of Dental Medicine,
**Department of Biochemistry, Faculty of Medicine,*
Medical University, Sofia

SUMMARY

The pulpo-dentinal complex has protective qualities safeguarding the dental pulp from progressive inflammations and traumatic lesions. This effect is achieved through the formation of a dentine-like structure made-up of cells capable of additionally differentiating. It is supposed that these are multipotent stem cells. The isolation and proving the existence of such cells is of crucial importance for regenerative medicine.

It is the aim of this project to isolate primary structures of mesenchymal stem cells from the pulp of temporary teeth.

The isolation of primary cells from the pulp of temporary teeth to be physiologically replaced was carried out. Immuno-fluorescence, analysis of the cell proliferation and a vitality test (MTT) were conducted too.

The project accomplished succeeded in isolating stem cell from the pulp of exfoliated temporary teeth. Such cells when in norm manifest no proliferation capacity.

The tooth morphogenesis is realised from the ectoderm amd mesoderm situated around the primordial mouth. The process is regulated through consecutive and reciprocal ecto-mesenchymal interactions along a couple of signal ways. The first result from them is the formation of the tooth bud that gives the start of the formation of the tooth. Following the realised consecutive cascades of proliferation and differentiation of the participating cells, the dental germ develops on to pass through a hat-like and a bell-like stadium, after which the hystogenesis of the tooth structures begins.

The interest towards the basic building cells of the dentine – the odontoblasts – remains to be big. These cells are unique in terms of origin and function. They originate from the neural combs and are a typical representative of the ectomesenchyme. They participate in the building of the organic matrix of the dentine and in its mineralisation. Contrary to the other building cells of the dental structures, they participate in the newly build dentine through their odontoblastic outgrowth, while the cell itself lives on the surface of the pulp. Thus they carry out an exchange within the dentine and contribute for the defence both of the dentine and the pulp. The finding of way for stimulating the existent odontoblasts, for improving the defence, as well for finding a way for differentiating the non-differentiated cells in the pulp to odontoblast-like cells is an important objective in the scientific researches.

Still not fully clear is the origin of the cells capable of undegoing a differentiation to odontoblast-like cells that can build up reparative dentine. It is supposed that under the odontoblasts a charge of daughter cells of preodontoblasts that have migrated from the neural combs remains. Not having a direct contact with the ectodermal cells that carry the embrionic stimuli these cells remain undifferentiated.

When the need of formation of a defence layer of dentine emerges, under the influence of certain stimulating products derived from the dysmineralised dentine, these cells could be stimulated to differentiate (5, 15). Besides, it is supposed that in the mature pulp stem cells are present that could undergo additional differentiation. Contemporary researches show that besides embryonal stem cells each adult organism has in many of its organs multipotent stem cells (5, 8, 15). They can serve as a sourse for differentiation of certain kinds of cells that can supersede damaged sectors of the tissues of an adult individual. These cells are a potential charge that could be used for a regeneration therapy. The proving of the presence of such cells and their isolation is a pretty difficult task (12). They have a relatively weak potential for differentiation and do not proliferate readily (5). Moreover, they are hard to isolate because of there only being a couple of them amongst thousands of other cells in the corresponding organ. When the researchers isolate and cultivate such cells, their proving to be of the required type is particularly important.

Usually cells from the pulp of wisdom-teeth are used (13).

Shi (14) makes the discovery that the pulp of the resorbed deciduos teeth has stem cells. These are postnatal stem cells that differ considerably in terms of qualities from the stem cells of adult individuals. They have the quality

to grow much more quickly and to double when cultivated. They are probably at a much less mature phase of development compared to the stem cells of adult individuals (14).

These discoveries increase the interest towards the stem cells from the pulp of deciduous teeth that can be stimulated to become odontoblasts or other dental cells, as well as cells from other organs and systems such as neural and masto-cystic cells. All these possibilities are in a stage of research and open new windows on the treatment and regeneration of tissues and organs (2, 4, 6, 10,17, 18, 19, 20, 21).

It is the **aim** of this research to isolate original mesenchymal stem cells from the pulp of deciduous teeth and to sustain the cell cultures thus obtained.

MATERIAL AMD METHODS.

1. Isolation of original mesenchymal stem cells from the pulp of deciduous teeth.

The experiment was conducted on routinely extracted – for a reason of their physiological change - deciduous teeth at the Department of Children's Dental Medicine, Faculty of Dental Medicine, Medical University, Sofia. Under aseptic conditions, cell cultures of mesenchymal stem cells were isolated from the pulp of these teeth, which was carried out at the Department of Biochemistry of the Faculty of Medicine, Medical University, Sofia.

The method of original cultivation through enzymatic separation by means of tripsine and colagenesis in a medium of DMED F12 (Dulbecco's Modified Eagle Medium/ Ham's F12) was applied. Immediately after the extraction, the deciduous tooth was treated with colagenesis V or tripsine and was left for 5 or 7 days in a medium of DMEM F12. Following that period the migrated pulp cells were tripsinised and sifted out into secondary cultures and grown in the same medium.

2. Immunofliorescence.

The cell cultures were grown on petri dishes and after reaching a subconfluent condition were fixated by means of 4% formaldehyde. The following antibodies were used for immunofluorescence: goat polyclonal anti-Alkaline Phosphatase antibody (RnD Systems), mouse monoclonal anti-C-kit antibody (Santa Cruz Biotech. Inc.), anti-Nestin antibody (Santa Cruz Biotech. Inc.), Donkey anti-Mouse TRITC conjugated antibody (Santa Cruz Biotech. Inc.), Donkey anti-Goat antibody (Santa Cruz Biotech. Inc.), Alexa Fluor 488 phalloidin и Sybr green NAS (Invitrogen).

RESULTS AND DISCUSSION

The isolation of primordial mesenchymal stem cells from the pulp of deciduous teeth was carried out through the filtration of the solution and through a subsequent centrifugation. After that a 10% FCS (fetal calf serum) and a 1% Penicillin/Streptomicine were added. The emergence of a confluence of the cells was waited for.

Fig. 1 shows the described primordial isolated mesenchymal cells which are not yet confluent. In Fig. 2 the subconfluent cell forms are presented, while Fig. 3 presents the final confluent mesenchymal cells from the pulp of a deciduous tooth.

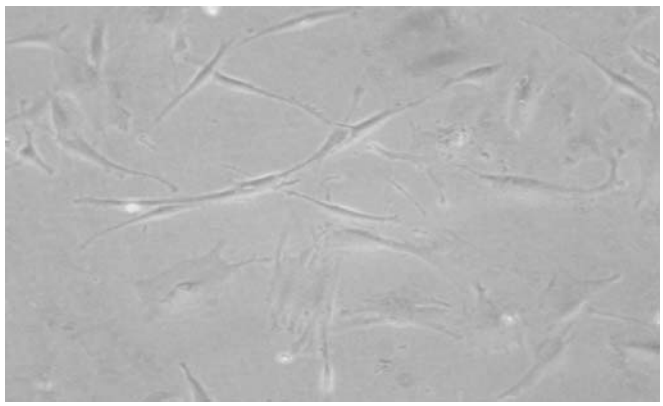

Fig. 1. Primordial pulp mesenchymal cells cultivated in a DMEM F12 cell medium with an addition of a 10% FCS and a 1% Penicillin/Streptomicine.

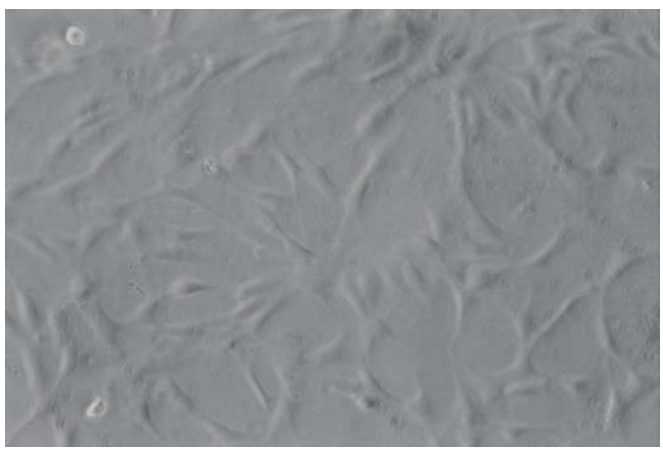

Fig. 2. Pulp mesenchymal cells, grown in a DMEM F12 cell medium with an addition of a 10% FCS and a 1% Penicillin/Streptomicine – subconfluent

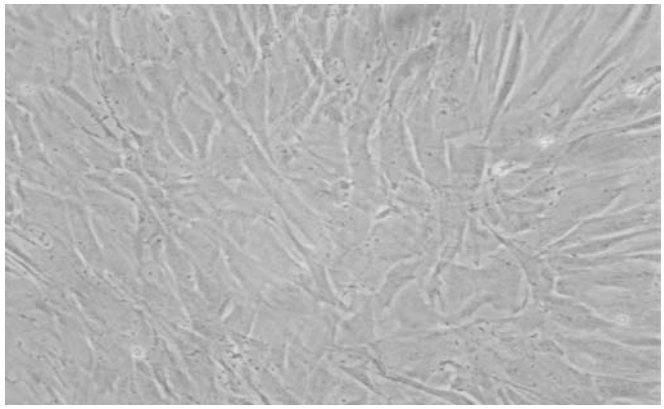

Fig. 3. Pulp mesenchymal cells, grown in a DMEM F12 cell medium with an addition of a 10% FCS and a 1% Penicillin/Streptomicine - confluent

Analysing the proliferative capabilities of the mesenchymal cells isolated from the pulp of a deciduous tooth (SHED) we found out that under autocrinic conditions and when grown in DMEM F12 without an addition of FCS and/or growth factors, the cells are of the weakly proliferative cell type (K in Fig. 4). The low proliferative status is probably due to the cells' function of a cell reserve in the dental pulp in vivo. Thus they remain as a potential charge of cells that are normally not used. The isolation carried out proved the presence of a similar cell reserve in the pulp of deciduous teeth.

In order that the real potential for proliferation of the determined cell is studied we added gradually increasing concentrations of FCS. Their introduction for 24 hours in the medium demonstrated a proliferative effect of the cells strongly conditioned by the amount of the added concentration (Fig. 4). The addition of just 1% FCS to the medium demonstrated an up to 7-time increase of the proliferative capability of the isolated cells. The addition of 10% FCS increased the proliferative capability up to 14 times, while a 30% content of FCS in the medium brought about a 20-fold increase of the DNA synthesis.

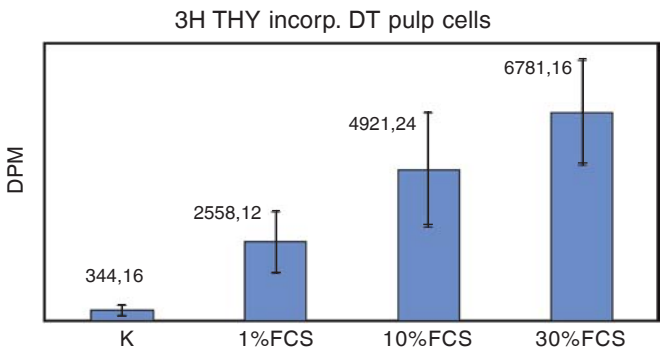

Fig. 4. Proliferative capabilities of the mesenchymal cells isolated from the pulp of a deciduous tooth (SHED).

These results show that the mesenchymal cells isolated from the pulp of a deciduous tooth while normally not evincing any proliferation capacity acquire and strongly increase this capacity depending on the concentration of the stimulating factors added, thus increasing the synthesis of DNA and becoming capable of cell proliferation.

Generalisation:

Stem cells present us with a tremendous perspective for developing regenerative medicine. A sourse of stem cells are also the umbilical cord of the neonate, the bone marrow, and lately some tissues and organs in the adult individuals. The problem of the isolation of the postnatal stem cells is in their quantity – just one cell per 100 000 cells in the corresponding tissue - as well as their identification, since they do not differ from the cells typical of the organ or tissue. Lately a potential of stem cells from the dental pulp of permanent teeth has been dioscovered. Extracted wisdom-teeth are used, as well as premolars extracted because of orthodontic reasons. This reserve of stem cells is of great interest to scientific research. In the last years it has been determined that such a potential of stem cells has the pulp of deciduous teeth. The possibility to isolate stem cells from teeth that are going to be shed for physiological reasons creates a new source of stem cells to be used in regenerative medicine. This is the most acceptable source of human postnatal stem cells which turned it into an important scientific object immediately after its discovery.

The realisation of this scientific potential demonstrated the presence of a cellular charge of stem cells in the pulp of deciduous teeth and the possibilities for their isolation and determination.

The existence of a potential of stem cells in the pulp of a deciduous tooth creates possibilities for a leap in the treatment of the early reversible phases of pulp inflammation.

CONCLUSION:

The isolation of stem cells from the pulp of exfoliated deciduous teeth was successful. This proved their presence even in the pulp of deciduous teeh in a period of reversible development. The non-differentiated cellular elements in the pulp of deciduous teeth look pretty similar to the embryonic structures. This cells normally do not evince proliferative capabilities. Additional researches are needed concerning the stimulation of the cell proliferation by means of different active products and growth factors. This would open new perspectives for the treatment of pulp inflammation and periodontal inflammation.

**** Grant project №35 of Medical University, Sofia, for 2007.***

BIBLIOGRAPHY:

1. Aida M, T. Irie, T. Aida, and T. Tachikawa. Expression of Protein Kinases C вI, вII, and VEGF during the Differentiation of Enamel Epithelium in Tooth Development *J Dent Res* 84(3):234-239, 2005

2. Brock D, R. Marty-Roix, and M. Spector. Smooth-muscle Actin in and Contraction of Porcin Dental Pulp Cells. J Dent Res, 81, 3, 2002, 203-208.

3. Camps J, I About, B Thonneman, T Mitsiadis et al. Two- Versus Three-Dimensional in vitro Differentiation of Human Pulp Cells into Odontoblastic Cells. Connective Tissue Research, 43, 293, 2004, 369-400.

4. Gagari E, M Rand, L Tayari, H Vastardis et all. Expression of stem cell facto rand its receptor, c-kit, in human oral mesenchymal cells. Eue J of Oral Sciences, 114, 5, 2006, 409-415.

5. Gilbert S. Developmental Biology, Seventh Edition, Sinauer AssociATES, Inc., Publishers, Sunderland, Massachusetts, 2003.

6. Iohara K, M. Nakashima, M. Ito, M. Ishikawa, A. Nakasima, and A. Akamine. Dentin Regenerationby Dental Pulp Stem Cell Therapy with Recombinant Human bone Morphogenetic Protein 2. *Dent Res* 83(8), 2004, 590-595.

7. Imad A. D Laurent-Maguin, U Lendahl, T Mitsiadis.Nestin expression in embryonic and adult human teeth under normal and pathological conditions. Am J Pathol. 2000 Jul;157(1):287-95.

8. Imad A. A Thimios. Molecular Aspects of Tooth Pathogenesis and Repair in vivo and in vitro Models *Adv Dent Res* 15:59-62, August, 2001 and Insulin-Like Growth Factor I Induce Bone Morphogenetic Proteins 2 and 4: A Mediator Role in Bone and Tooth Formatin? Endocrinology. 139, 9, 1998, 3855-3862.

9. Li H, P Bartold, C Zhang, R Clarkson, W Young, M Waters. Growth Hormone and Insulin-Like Growth Factor I Induce Bone Morphogenetic Proteins 2 and 4: A Mediator Role in Bone and Tooth Formation? Endocrinology, 139, 9, 1998, 3855-3862.

10. Luukko K. Neuronal cells and neurotrophins in odontogenesis. *Eur J Oral Sci* 1998; 106 (suppl 1): 80–93. © Eur J Oral Sci, 1998

11. Mark M, A Bloch-ZupanL, J Ruch. Effects of retinoids on tooth morphogenesis and cytodifferentiation *in vitro Int J Dev Biol* 1992; 36: 517–526

12. Markku Larmas. Odontoblast Function Seen as the Response of Dentinal Tissue to Dental Caries *Adv Dent Res* 15:68-71, August, 2001

13. Martin A, F Unda, C Begue-Kirn, J Ruch, J Arechaga. Effects of aFGF, bFGF, TGFb1 and IGF-I on odontoblast differentiation in vitro. *Eur J Oral Sci* 1998; 106 (suppl 1): 117–121. © Eur J Oral Sci, 1998

14. Miura M, S Gronthos, M Zhao, B Lu, L Fisher, P Robey, S Shi SHED: Stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A. 2003 May 13; 100(10): 5807–5812.

15. Мьller W. Developmental biology, Springer, 1996, 237-241, 263-280

16. Nanci A. Ten Cate's Oral Histology, Development, Structure, and Function, sixth Edition,Mosby, 2003,

17. Stokowski A, S Shi, T Sun, P Batold et all. EphB/Ephrin-B Interaction Mediats Adult Stem Cell Attachment, Spreading, and Migration: Implications for Dental Tissue.

18. Takeyasu M, I Nazaki, M Daito. Differentiation of dental pulp stem cells into a neural lineage. Pediatric Dental Journal, 16, 2, 2006, 154-162.

19. Thesleff I, A Vaahtokari, A Partanen. Regulation of organogenesis. Common molecular mechanisms regulating the development of teeth and other organs. *Int J Dev Biol* 1995; 39: 35–50.

20. Tureckova J, C Sahlberg, T Berg, J Ruch, I Thesleff, R Peterkova. Comparison of expression of the msx-1, msx-2, BMP-2 and BMP-4 genes in the mouse upper diastemal and molar tooth primordia. *Int J Dev Biol* 1995; 39: 459–468.

21. Zhang J, X Duan, H Zhang, Z Deng et all. Isolation of neural crest-derived stem cells from rat embryonic mandibular processes. Biology of the Cell, 98, 2006, 567-575.

Adress for corespondense:
Milena Peneva
Department of Pediatric Dentistry, Faculty of Dental Medicine,
1, Georgi Sofiiski str., 1431 Sofia, Bulgaria
E-mail: milenapeneva@mail.bg