# Effect of basic fibroblast growth factor on the gene expression of decorin in periodontal ligament cells★

Cheng Yuan[1], Zeng Zhao-fang[1], Zhu Jun[2]

[1]Chongqing Medical University, Chongqing 400016, China; [2]School of Civil Engineering, Southwest Jiaotong University, Chengdu 610031, Sichuan Province, China

Cheng Yuan★, Master, Chongqing Medical University, Chongqing 400016, China

Correspondence to: Zeng Zhao-fang, Professor, Chongqing Medical University, Chongqing 400016, China zeng000@126.com

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**Abstract**

**OBJECTIVE:** Studies have confirmed that basic fibroblast growth factor stimulating periodontal ligament cells can promote the proliferation of human periodontal ligament cells, so as to facilitate the reconstruction of lost periodontal tissues. By use of different concentrations of basic fibroblast growth factor, the *in vitro* cultured normal human periodontal ligament cells were stimulated to observe the decorin gene expression in the periodontal ligament cells.

**METHODS:** The periodontal ligament cells were isolated and cultured using trypsin digestion method. The third generation of cells at logarithmic growth phase were preserved in DMEM containing 10% DMSO and 20% FBS frozen cryopreservation fluid, then moved into liquid nitrogen preservation at the second day. Immunohistochemistry was used to identify anti-vimentin and cytokeratin staining. The sixth generation of human periodontal ligament cells were divided into experimental group and control group. The experimental group was cultured in DMEM culture medium containing basic fibroblast growth factor at the concentration of 0.1, 1, 10 μg/L under standard conditions for 24 hours; control group was cultured with DMEM culture medium under standard conditions for 24 hours. The intracellular decorin gene expression was determined using RT-PCR.

**RESULTS:** By microscopic observation, the cells in the control group had no significant changes, and cells proliferated in the experimental group, before stimulation the cells at the bottom of the bottle became denser than those in sparse regions. Adding basic fibroblast growth factor into periodontal ligament cells, could significantly decrease the mRNA expression of decorin, along with the increase of basic fibroblast growth factor concentration, the inhibition effect gradually weakened and became the strongest in the 0.1 μg/L but the weakest at 10 μg/L.

**CONCLUSION:** The basic fibroblast growth factor stimulation can promote the proliferation of periodontal ligament cells and inhibit the synthesis of decorin in a dose-dependent manner, 0.1 μg/L induces the strongest inhibitory effect.

## INTRODUCTION

Periodontitis is a kind of chronic progressive diseases with periodontal tissue loss as the main manifestations, clinical manifestations include gingival inflammation, periodontal pocket formation, alveolar bone absorption and loose teeth, displacement. After periodontal basic therapy, surgical treatment combined with drug therapy and occlusal treatment, the inflammation of periodontal tissues can be controlled, but the loss of periodontal tissue is irreversible. The clinical regenerative surgery commonly used are mainly guided tissue regeneration and bone graft, a single treatment for periodontal tissue regeneration has its own advantages, but also has its limitations. Among four kinds of source cells for wound healing in periodontal tissues, only the periodontal ligament cells have the ability to regenerate periodontal tissue[1]. Basic fibroblast growth factor (bFGF) exhibits various biological effects on periodontal ligament cells, it can promote the proliferation of periodontal ligament cells, promote hyaluronic acid synthesis, increase laminin mRNA levels, inhibit type Ⅰ collagen fibers, alkaline phosphatase and tropoelastin synthesis[2]. The aim of this study is to reconstruct the lost periodontal tissues using bFGF stimulation.

## MATERIALS AND METHODS

**Design:** Controlled observation cytological observation.

**Materials:** Health premolar was harvested by orthodontic extraction from 12 to 18-year-old adolescents who were admitted to Out-patient Department of Oral Surgery. All donors and their relatives were informed of and gave consents to the collection of materials.

### Methods

***Harvest, culture and amplification of human normal periodontal ligament cells***

Primary culture[3]: The Health premolar was immediately thrown into the penicillin packaging bottle containing sterile D-Hanks solution, and gently shaken about 5–10 minutes to clear the blood on the tooth root, then the teeth were transferred into another penicillin packaging bottle containing sterile D-Hanks solution by using sterile tweezers, and finally preserved at the laboratory under ice bath.

Serial subcultivation: When the cells reached 80%–90% confluence, we aspirated the medium, digested with the mixture of 0.25% trypsin and 0.02% EDTA (1:1) for 2–5 minutes, and the digestion was terminated when the cell neurite retracted and the cells became round by microscope. By pipette repeated blowing, the cell suspension was formed, without cell mass left. The cell suspension was vaccinated in 1:2 ratio into 25-cm$^2$ culture sterile bottle, and he culture medium was renewed every 3 days.

Cell cryopreservation: The third generation of logarithmic phase cells were used, changed the medium one day before cryopreservation. The cells at monolayer growth were digested down using 0.25% trypsin, and then prepared into cell suspension with pipette gentle and repeated blowing. Cell suspension was transferred into 50-mL sterile

centrifuge tube, centrifuged at 800 r/min for 10 minutes. The supernatant was aspirated carefully, added 1.5 mL cell cryopreservation liquid, that is DMEM containing 10% DMSO and 20% FBS. After mixed well enough, cell suspension was transferred to 1.5-mL sterile cryopreservation pipes, labeling the name of cells and the cryopreservation time, and then put in -80 ℃ overnight, at last preserved in liquid nitrogen at the second day.

***Identification of human normal periodontal ligament cells***

Preparation of cell glass coverslips: The coverslips were rinsed according to the requirements of cell culture and high-pressure steam sterilizer, followed by a steam sterilization, then placed into six-well plates. The cell suspension was cultured in the six-well plates, after the cells adhered, they were cultured conventionally. The culture medium was absorbed when the cells reached 70%–80% confluence, washed three times using PBS, 30 seconds once each time. Adding 4 g/L paraformaldehyde for fixation 30 minutes, washing with PBS for another three times, 3 minutes each time. After drying, the cell-free plane of the coverslips was glued to the microscopic slide, then dried at room temperature and reserved at room temperature.

Anti-vimentin and cytokeratin staining by immunohistochemistry: The procedure was performed in accordance with the SP kit instructions.

Stimulation of human periodontal ligament cells cultured in vitro by exogenous bFGF: The sixth generation of human periodontal ligament cells were divided into experimental group and blank control group, with three bottles of cells in each group. The experimental group was cultured with DMEM culture solution containing bFGF of 0.1, 1, 10 μg/L respectively for 24 hours, while the control group only with DMEM culture solution for 24 hours.

Detection of the decorin mRNA level by RT-PCR[4]:

The primer sequences of decorin are as follows:

Upstream primer: 5'-GTC TGG ACA AAG TGC CAA AGG A-3'

Downstream primer: 5'-ATC AGC AAT GCG GAT GTA GGA G-3'

The primer sequences of β-actin are as follows:

Upstream primer: 5'-GGC ATC GTG ATG GAC TCC C-3'

Downstream primer: 5'-TCG CTG TCC ACC TTC CAG C-3'

Measurement of the absorbance value and area of the electrophoresis strip: The absorbance value and area of each band electrophoresis strip was detected by GIS gel analysis system, and then recorded.

**Main outcome measures**

The changes of decorin gene expression in cells.

**Design, enforcement and evaluation**

All authors have designed, implemented and assesses this study.

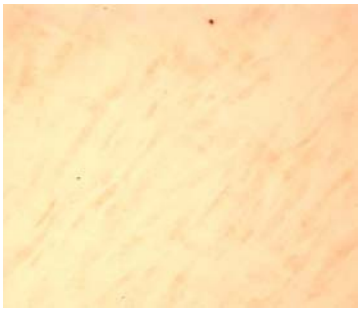
**Statistical analysis**

The analysis was performed by the first author and corresponding author using SPSS 13.0 software package. The measurement data were expressed as Mean±SD. By use of variance analysis, a level of $P < 0.05$ was considered statistically significant.

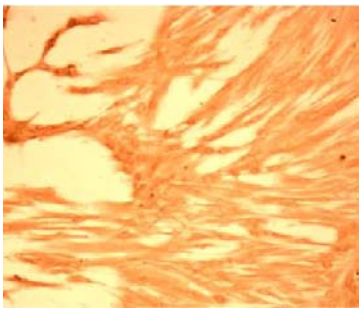
## RESULTS

**Identifications of human normal periodontal ligament cells**

The results showed that human normal periodontal ligament cells were negative for anti-keratin staining and positive for anti-vimentin staining. Both stainings were negative in the control group (Figures 1, 2).



a: Anti-keratin staining



b: Anti-vimentin staining

Figure 1 Anti-keratin staining and anti-vimentin staining of human normal periodontal ligament cells (×100)

Figure 2 Negative control identification of human normal periodontal ligament cells (×100)

**Exogenous bFGF stimulates the morphology of human normal periodontal ligament cells**

By microscopic observation, the morphology of cells in the control group had no significant changes, and cells were shown to proliferate in the experimental group, before stimulation the cells at the bottom of the bottle became denser than those in sparse regions.

**RT-PCR detection of decorin gene expression in cells**

The electrophoresis analysis of PCR products showed an electrophoresis band at about 416 bp and 613 bp in both groups, and the band brightness in target segment of the experimental group was obviously reduced compared with control group, this result was consistent with the expected results.

The measured absorbance values and the areas of various electrophoretic bands were introduced into the formula: ratio = decorin (absorbance value × area value) / β-actin (absorbance value × area value) for calculation (Table 1).

Table 1 Detection of decorin gene expression in human periodontal ligament cells by RT-PCR ($\bar{x}\pm s$)

| Group | Ratio |
|---|---|
| Experimental | |
| 0.1 μg/L | 0.60±0.12 |
| 1 μg/L | 0.86±0.16 |
| 10 μg/L | 0.90±0.20 |
| Blank control | 1.19 |
| *F* | 4.985 |
| *P* | 0.019 |

The mRNA expression of decorin declined after the periodontal ligament cells were stimulated by bFGF (*P* =0.019), and the inhibition effect of the bFGF on the decorin gene expression could decline as the concentration raised. In the 0.1 μg/L concentration, it had the strongest inhibitory effect, while in the 10 μg/L it had the weakest.

## DISCUSSION

**Fibroblasts**

Fibroblast, which is also called periodontal ligament cells, is the most common cells in periodontal ligament. Its main function is collagen synthesis, at the same time swallowing and degrading the old collagen fibers[1]. The function of periodontal ligament cells decides its importance in the regeneration of periodontal tissue.

In our experiment, we obtained periodontal ligament cells from premolar periodontal membrane of healthy adolescents by orthodontic extraction, which was easy to get and fresh periodontal tissues. Although the number of periodontal ligament cells in pericementum is not large, it has many advantages for scientific research, such as easy for primary culture and amplification *in vitro*, no pollution of other cell lines, stable biological characters, mature culture technology and so on. Therefore, it is a good way for studying the role of bFGF on periodontal ligament cells through culturing them *in vitro*.

**The role of bFGF on periodontal ligament cells**

Immunohistochemical staining showed that bFGF exists in the periodontal ligament fibroblasts, endothelial cells and some fibroblasts, which can synthesize and secrete bFGF by themselves, and begin to store bFGF from the stage of fibroblast cells. The bFGF distributes in the cytoplasm and nucleus of periodontal ligament cells. As bFGF growth factor can promote the proliferation of periodontal ligament cells, it can induce the growth of periodontal tissue[5]. What's more, bFGF promotes the proliferation of human gingival fibroblasts, human periodontal ligament fibroblasts and human alveolar bone cells, just having different concentrations for the best effect[6].

For many substances related to the regeneration of periodontal tissues, such as alkaline phosphatase and hyaluronic acid, bFGF plays a role of either promoting or inhibiting them. This study shows that the numbers of hyaluronic acid molecules in periodontal ligament cells cultured with the conditioned medium adding bFGF were much larger than that cultured in the medium without bFGF. The mRNA expression level of hyaluronan synthase 1 and 2, both of which are involved in the synthesis of hyaluronic acid, had been enhanced as detected by RT-PCR. These results indicate that bFGF is involved in the regulation of hyaluronic acid's synthesis, the maintenance of homoeostasis and the regeneration of periodontal tissues.

As for the gene expression of type Ⅰ collagen and metalloprotease-1, bFGF has the reverse time and dose dependence, it can reduce the gene expression of type Ⅰ collagen as well as increase the metalloproteinase-1 gene expression at the same time, but no such affection on collagen Ⅲ. The influence of bFGF on these three gene expressions can be regulated by the culture time in the bFGF-contained medium. These results show that bFGF is one of the important regulators for the effective reconstruction of collagen I in pericementum. The bFGF may also have the same function to other connective tissue.

**Influence of bFGF on decorin**

As a major proteoglycan in periodontal tissue, decorin distributes in collagen fiber bundles at the basement membrane zone of gingival epithelial as well as the gingival and periodontal connective tissue. Stronger immune expression was observed in the subepithelial gingival than in the deep periodontal region. Decorin can bind different types of collagen fibers, such as collagen Ⅰ and collagen Ⅵ, owing to its horseshoe-shaped core protein[7]. Therefore, it was considered to be impacting the collagen synthesis and its final diameter.

Our experiment shows that the mRNA expression level of decorin in periodontal ligament cells was obviously reduced with the presence of bFGF. Moreover, the inhibitory effect gradually weakened with the increased concentration of bFGF, the strongest inhibitory effect appeared in the 0.1 μg/L, while the weakest in the 10 μg/L. The following points discussed are its significance: ① The inhibition effect of bFGF on decorin has changed the homeostasis of the extracellular matrix, decreasing the synthesis of collagen I. Decorin is an important factor regulating collagen degradation, and bFGF may also regulate collagen synthesis through this way. ② In the teeth and periodontal tissues, the small interstitial proteoglycan expresses in dentin and the non-mineralized areas of cementum. Especially the collagen fibers from the cementum and alveolar bone inserting at the junction of soft and hard tissue, and the osteoblasts in the inner alveolar bone showed strong positive immunohistochemical characteristics, indicating that small interstitial proteoglycan may affect the mineralization of dentin, cementum and alveolar bone. ③ Decorin can mediate cell proliferation, its expression increases in the dormancy stage, while is very low in the active proliferation of cells. ④ Decorin plays an important role in the process of growth, development

and damage repairs, and also participates in the tissue stability and damage repairs. The inhibition effect of bFGF on the decorin indicates that it may be involved in the inflammatory process of periodontal disease and bFGF plays a great role on the decorin gene expression in periodontal ligament cells, providing a theoretical basis for promoting the pluripotent cells functions of periodontal ligament cells in regeneration of periodontal tissues.

In short, the periodontal tissue regeneration is a very complex process. However, the inhibition of bFGF on the decorin synthesis is one of the important regulators of promoting periodontal ligament cells proliferation. Application of bFGF can effectively induce regeneration of periodontal tissues, not only inducing the connective tissue regeneration, but also inducing the bone regeneration and alveolar bone regeneration, having a broad application prospect.

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# 碱性成纤维细胞生长因子对牙周膜细胞内核心蛋白多糖基因表达的影响*

程　远[1]，曾照芳[1]，朱　俊[2] ([1]重庆医科大学，重庆市　400016；[2]西南交通大学土木工程学院，四川省成都市　610031)

**程　远★**，女，1981 年生，河北省衡水市人，汉族，重庆医科大学毕业，硕士，主要从事生物医学信息学研究。

**通讯作者**：曾照芳，教授，重庆医科大学，重庆市　400016

**摘要**

**目的**：研究已证实，碱性成纤维细胞生长因子刺激牙周膜细胞后可促进人牙周膜细胞的增殖，以利于重建丧失的牙周组织。利用不同质量浓度碱性成纤维细胞生长因子刺激体外培养的人正常牙周膜细胞，观察牙周膜细胞内核心蛋白多糖基因表达。

**方法**：采用胰酶消化法分离培养牙周膜细胞。取第 3 代对数生长期的细胞，加入含有 10%二甲基亚砜和含体积分数 20%胎牛血清冻存液的 DMEM 中进行冻存，第 2 天移入液氮中保存。免疫组织化学方法行抗波形丝蛋白、角蛋白染色鉴定。取第 6 代人牙周膜细胞，分为实验组和空白组。实验组分别用含质量浓度为 0.1，1，10 μg/L 碱性成纤维细胞生长因子的 DMEM 培养液标准条件下培养 24 h；空白组用 DMEM 培养液标准条件下培养 24 h。RT-PCR 法检测细胞内核心蛋白多糖基因表达变化。

**结果**：镜下观察空白组细胞未见明显变化，实验组可见细胞增殖，刺激前瓶底细胞较稀疏的区域变得密集了。加入碱性成纤维细胞生长因子的牙周膜细胞内核心蛋白多糖的 mRNA 表达水平明显下降了，而且随着碱性成纤维细胞生长因子质量浓度的增加，抑制作用逐渐减弱，在 0.1 μg/L 时抑制作用最强，在 10 μg/L 时抑制作用最弱。

**结论**：碱性成纤维细胞生长因子刺激可促进牙周膜细胞增殖，呈剂量依赖性抑制核心蛋白多糖的合成，0.1 μg/L 时抑制作用最强。

**关键词**：牙周组织再生；碱性成纤维细胞生长因子；核心蛋白多糖；组织工程

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