JOURNAL OF BIOLOGICAL REGULATORS & HOMEOSTATIC AGENTS
Volume 34, No. 1 (Supplement 2), January-February 2020
SPECIAL DENTAL ISSUE

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COLLAGENATED HETEROLOGOUS CORTICO-CANCELLEUS BONE MIX STIMULATED DENTAL PULP DERIVED STEM CELLS

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Collagenated heretologous cortico-cancelleus bone mix (CHCCBM) is largely employed in maxillary and dental surgery for regeneration procedures, and is similar to human bone from chemical and physical point of view and promotes osteogenesis. In order to get more inside how this biomaterial induces osteoblast gene expression to promote bone formation, the mRNA levels of bone related genes were compared in human osteoblasts and dental pulp stem cells, using real time RT-PCR. The obtained results demonstrated that CHCCBM enhance stem cells differentiation and deposition of matrix by the activation of osteoblast related genes SP7, FOSL1 and SPP1.

Collagenated heretologous cortico-cancelleus bone mix (CHCCBM) is a bovine bone derived employed in maxillary and oral surgery. Some reports have demonstrated its clinical efficacy to restore alveolar ridge in pre-prosthetic surgery (1-3).

To investigate the molecular mechanism by which CHCCBM promotes osteoblast differentiation and proliferation, the quantitative expression of the mRNA of specific bone related genes, was examined in derived dental pulp stem cells treated CHCCBM. Dental pulp stem cells (DPSCs) represents an ideal source of stem cells because approachable niches containing a high number of stem cells compared to equal volumes of bone marrow (4-6). In this study the expression levels of specific genes were examined by means of real time RT-PCR in DPSCs after treatment with CHCCBM.

Gene expression in DPSCs was then compared with the gene expression in treated Human Osteoblasts (HOb) to evaluate the potential effect of this material in osteoblasts differentiation.

MATERIALS AND METHODS

Stem cells isolation from dental pulp, flow cytometric analyses and primary human osteoblasts cell culture were performed as previously described (4-6).

Cell treatment

DPSCs and HOb were seeded with CHCCBM (Gen-Os, Tecnoss srl, Giaveno, Torino, Italy) at the concentration of 10 mg/ml. Another set of wells containing untreated cells were used as control. The cells were maintained in a humidified atmosphere of 5% CO2 at 37°C. Cells were harvested at two time points, 15 and 30 days, for RNA extraction. RNA processing, RT-PCR and statistical analyses were conducted as previously described (4-6).

Key words: bone, dental pulp, stem cells, gene expression, osteoblast differentiation

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0393-974X (2020)

RESULTS

Cell cultures were phenotipically characterized by flow cytometric analyses as previously reported (4-6). To study if CHCCBM stimulates osteoblasts differentiation and proliferation in DPSCs, several osteoblast genes and mesenchymal stem cells marker, were analyzed by quantitative real-time PCR after 14 days of treatment with CHCCBM. Treated DPSCs resulted in up-regulation of SP7, FOSL1 and SPP1 genes and down-regulation of ENG, RUNX2, COL3A1, COL1A1 and ALPL during the first 14 days of treatment.

Analyzing the results obtained in HOb, we observed that after 14 days of treatment SP7, ENG, FOSL1, COL1A1, COL3A1 and SPP1 were up-regulated. The expression of RUNX2 and ALPL was down-regulated. "Two tailed ANOVA" comparison between DPSCs and HOb after the treatment showed that all genes were significantly differentially expressed after 14 days of treatment. (Table I).

DISCUSSION

CHCCBM (Gen-Os, Tecnoss srl, Giaveno, Torino, Italy) is widely used in several bone regeneration procedures in oral surgery due to its characteristics of resorption rate and capability of promotes osteogenesis. It is of paramount importance since several situation determine bone loss. Among them are socket preservation after tooth extraction, peri-implants bone maintenance (7-16), ridge reconstruction in syndromic patients (17-23) and oral rehabilitation after oncological treatments (24-35)

In order to get more inside how CHCCBM acts on DPSCs, changes in expression of bone related marker genes (RUNX2, SPP1, SP7, COLIA1, COL3A1, ALPL and FOSL1) and mesenchymal stem cells marker (ENG) were investigated by real-time RT–PCR. DPSCs were isolated by enzymatic digestion and phenotipically characterized by flow cytometric analyses. Dental pulp derived cell were homogenously $CD105^{+}$, $CD90^{+}$, $CD34^{-}$, $CD45^{-}$, $CD14^{-}$, which is a typical mesenchymal stem cells surface antigen profile.

The osteoinductive properties of CHCCBM were demonstrated by the up-regulation of SP7 during all the entire treatment both in treated DPSCs than in treated HOb. SP7 is a transcriptional factor involved in bone formation and osteoblast differentiation downstream of RUNX2 pathway. CHCCBM also modulate the expression of collagenic extracellular matrix genes, collagen type 1α1 (COL1A1) and collagen type 3α1 (COL3A1).

After 14 days, in treated DPSCs was observed the

Table I. *Differentially expressed genes between DPSCs and HOb after 14 days of treatment.*

Genes	**DPSCs**	**HOb**	**Differentially expressed genes**
	Log10 RQ	**Log10 RQ**	**p<0,005**
SP7	1,22	0,52	**0,001**
ENG	-0,42	0,03	**0,009**
FOSL1	0,25	0,26	**0,001**
RUNX2	-0,42	-0,03	**0,002**
COL3A1	-1,46	0,02	**0,001**
COL1A1	-0,71	0,13	**0,001**
ALPL	-0,54	-0,23	**0,001**
SPP1	0,93	1,64	**0,001**

down-regulation of COL1A1 and COL3A1. Instead, in HOb, the two collagens type were up-regulated. Osteopontine (SPP1) was up-regulated during all the treatment both in DPSCs than in HOb. The up-regulation of this gene suggests the differentiation effect of treatment.

ENG, also named CD105, a surface marker used to define a bone marrow stromal cell population capable of multilineage differentiation was down-regulated in DPSCs and up-regulated in HOb during the entire treatment. Another gene involved in osteoblast differentiation and modulated by CHCCBM was FOSL1. Both in DPSCs than in HOb this gene was up-regulated. FOSL1 is a component of the dimeric transcription factor AP-1 involved in the transcription of bone related genes. This study demonstrates that CHCCBM is capable of supporting osteoblast differentiation and extracellular matrix deposition and mineralization in mesenchymal stem cells by the activation of osteoblast related genes SP7, SPP1 and FOSL1.

Within the limits of this *in vitro* study related to the period of observation (2 weeks), it possible to infer that CHCCBM is a bone substitute that positively active gene related to bone formation.

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SURVIVAL AND SUCCESS RATE OF SPIRAL IMPLANTS INSERTED MANDIBLE

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The rehabilitation of the edentulous mandible is a relatively common clinical problem and dentatl implants are popular medical device routinely use in daily practice. Recently a new type of two-piece spiral implants has been introduced in the market. Here a retrospective study is reported. A total of 54 two-piece implants were inserted in mandible in the period between June and December 2017, 30 in female and 24 in males. The median age was 53 ± 8. Implants replaced 11 incisors, 6 cuspids, 23 premolars and 14 molars. Implant' length was 10 mm, 11,50 mm and 13 mm in 16, 19 and 19 cases, respectively. Implant' diameter was 3.3 mm, 3.75 mm and 4.2 mm in 22, 13, 19 cases, respectively. Twenty two fixtures were placed in totally edentulous patient and 32 in partially edentulous subjects. There were 4 single crowns, 28 implants bearing two or greater bridges, 4 removable dentures and 18 supporting Toronto bridge. The overall mean follow-up was 13 ± 2 months. One implant was lost so that survival rate (SVR) was 98.15%. Then peri-implant bone resorption (success rate, SCR) was used to investigate peri-implant bone stability. No implant have a crestal bone resorption greater than 1.5 mm so that the implants studied are reliable devices for oral rehabilitation with a very high SCR and SVR.

Dental implant rehabilitation is a safe and well-documented procedure. Dental implants have been shown to provide a reliable basis for fixed and removable prostheses (1). Several techniques have been proposed for use of dental implants in retention and stabilization of denture in mandible. Also in case of extremely resorbed mandible implants can be inserted with two procedures (2): use of (short) endosseous implants or augmentation of the mandible by means of grafting procedures, followed by the insertion of endosseous implants with either a fixed or removable prosthesis.

Knowing the ideal conditions for peri implant bone regeneration has led to more refined concepts of implant loading in that secure primary stability and solid bone quality are considered to form a sound basis for early or immediate loading protocols (3). This recruitment is based on a cascade of events that starts with adsorption of biologically active molecules such as fibrin and thrombin as well as the attachment of platelets to the implant surface (4). Surface modifications of implants which enhance these initial steps could improve the recruitment of bone cells and shorten the critical period of peri-implant bone formation, thereby decreasing the risk of implant loosening during early loading.

Today, important improvements have been made in understanding the prosthodontic aspects of implant-related treatment (5). Now it is possible to provide retention for fixed and removable prostheses.

Key words: two-pieces, implant, fixture, bone, loading

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0393-974X (2020)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

This kind of treatment improves oral function and patient satisfaction. However, the use of implants in the extremely resorbed mandible due to neoplasm (6-17), malformations (18-24) and periodontal diseases (25-34) are still subjects of discussion in the literature.

Since a new type of fixture (Tuff type, Noris Medical Ltd. Nesher, Israel) has been introduced recently in the market, we analyses a series of two-pieces implants inserted in lower jaw in order to evaluate their survival (i.e. total number of fixtures still in place at the end of the follow-up) and success rate (i.e. peri-implant bone resorption).

MATERIALS AND METHODS

Study design/sample

To address the research purpose, the investigators designed a retrospective cohort study. Subjects were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity; in addition, the patients had to agree to participate in a post-operative check-up program. The exclusion criteria were as follows: bruxists, consumption of alcohol higher than 2 glasses of wine per day, localized radiation therapy of the oral cavity, antitumor chemotherapy, liver, blood and kidney diseases, immunosupressed patients, patients taking corticosteroids, pregnant women, inflammatory and autoimmune diseases of the oral cavity.

Variables

Several variables are investigated: demographic (age and gender), anatomic (tooth site, jaws), implant (length, diameter) and prosthetic (i.e. single crowns, bridges, removable dentures and Toronto bridge.) variables.

The predictors of outcome are the percentage of implants still in place at the end of the follow-up period (i.e. survival rate – SVR) and the peri-implant bone resorption. The latter is defined as implant success rate (SCR) and it is evaluated according to the absence of persisting peri-implant bone resorption greater than 1.5 mm during the first year of loading and 0.2 mm/years during the following years.

Data collection methods

Before surgery, radiographic examinations were done with the use of intra-oral radiographs and orthopantomographs. Peri-implant crestal bone levels were evaluated by the calibrated examination of intra-oral radiographs and orthopantomograph x-rays after surgery and at the end of the follow-up period. The measurements were carried out medially and distally to each implant, calculating the distance between the implant's neck and the most coronal point of contact between the bone and the implant. The bone level recorded just after the surgical insertion of the implant was the reference point for the following measurements. The measurement was rounded off to the nearest 0.1 mm. The radiographs were performed with a computer system By knowing dimensions of the implant, it was possible to establish the distance from the medial and distal edges of the implant platform to the point of bone-implant contact (expressed in tenths of a millimeter) by doing a proportion. The difference between the implant-abutment junction and the bone level was defined as the Implant Abutment Junction (IAJ) and calculated at the time of operation and at the end of the follow-up.

Surgical protocol

All patients underwent the same surgical protocol. An antimicrobial prophylaxis was administered with 1g Amoxycillin 500 mg twice daily 5 days starting 1 hour before surgery. Local anesthesia was induced by infiltration with articaine/epinephrine and post-surgical analgesic treatment was performed with 600 mg Ibuprofen twice daily for 3 days. Oral hygiene instructions were provided. Two-piece implants (Tuff type, Noris Medical Ltd. Nesher, Israel) were inserted in the period between June and December 2017. The implant neck was positioned at the alveolar crest level. A second operation was then performed after four months to loading by means a provisional prosthesis. The final restoration was usually delivered within 8 weeks. All patients were included in a strict hygiene recall.

Data analysis

Pearson-chi square test was used to detect those variables statistically associated to SVR and SCR.

RESULTS

A total of 54 two-piece implants (Tuff type, Noris Medical Ltd. Nesher, Israel) were inserted in mandible in the period between June and December 2017, 30 in female and 24 in males. The median age was 53 ± 8. Implants replaced 11 incisors, 6 cuspids, 23 premolars and 14 molars. Implant' length was 10 mm, 11,50 mm and 13 mm in 16, 19 and 19 cases, respectively. Implant' diameter was 3.3 mm, 3.75 mm and 4.2 mm in 22, 13, 19 cases, respectively. Twenty-two fixtures were placed in totally edentulous patient and 32 in partially edentulous subjects. There were 4 single crowns, 28 implants bearing two or greater bridges, 4 removable dentures and 18 supporting Toronto bridge. The overall mean follow-up was 13 ± 2 months. One implant was lost, survival rate (SVR) = 98.15%. Then peri-implant bone resorption (success rate, SCR) was used to investigate peri-implant bone stability. No implant have a crestal bone resorption greater than 1.5 mm.

DISCUSSION

Implant therapy is considered a predictable treatment option rendering high implant and prosthesis survival rate when using fixed dental prostheses for replacing single- and multiunit gaps (35). The primary goal following implant placement is to achieve osseo-integration, a solid anchorage of the endosseous part of the implant within the bony envelope. Various methods have been developed to measure osseo-integration and implant stability, including resonance frequency analysis and histologic analyses (36).

The removable prosthesis is supported by both implant and mucosa and generally requires fewer implants when compared with the totally implant supported prosthesis design. Fewer implants and a removable prosthesis offer a less complex and less expensive option for an edentulous patient (37). In terms of reducing patient complaints, Cune et al.(38) suggested that treatment with dental implants in combination with an overdenture is very effective. Considering mandible implants, patients comfort was strongly improved.

Nowadays, computed tomography (CT) is an excellent tool for immediate loading procedures in implant dentistry to reduce risks to the patients, to prevent breaches of planning and assisting in the placement of implants in guided surgery. Computed tomography is an accurate, non-invasive technique that assists the practitioner in improving the accuracy of the positioning of the implants. Wittwer et al. (39) after an evaluation of immediate loading using computer-guided flapless surgery, concluded that trans-mucosal computer- assisted placement and immediate loading of mandibular implants is a high-end approach to edentulism that provides excellent results while being minimally invasive. The goal of this advancement is not only to improve the precision and predictability of implant placement but also to change an invasive protocol for a flapless surgical procedure (non-invasive) and an immediate loading. Margonar et al. (40) consider this technique a better planning of the implants, that makes the procedures more accurate, reduces surgical time, and leaves the patient more satisfied.

In the present report only one implant was lost with a survival rate of 98.15%. Then peri-implant bone resorption (i.e. delta IAJ) was used to investigate SCR but no variable statistically affected implant outcome. In conclusion the studied implants (Tuff type, Noris Medical Ltd. Nesher, Israel) are reliable devices for oral rehabilitation with a very high SCR and SVR.

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IMPLANTS OUTCOME INSERTED IN DIFFERENT SITES

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Oral rehabilitation by means dental implants has high standards of success. Recently, a new type of two-pieces spiral implants has been introduced in the market. Since few reports focus of the efficacy of this medical device as a reliable tool for oral rehabilitation, here a retrospective study is reported. In the period June-December 2017 one hundred and two spiral fixtures were inserted, half in females and 51 in males. The median age was 56 ± 8 (min-max 36-73 years). Forty-eight implants were inserted in upper jawbone and 54 in mandible. Two implants were lost and thus survival rate (SVR) is 99.9%. Then peri-implant bone resorption was used to investigate the clinical success (success rate, SCR) over time. No implants have a crestal bone resorption greater than 1.5 mm in the first year follow up. No studied variable has an effect on clinical outcome. In conclusion the studied implants have high SCR and SVR so that they are good tools for oral rehabilitation.

Dental implants are used to restore missing tooth (1). When dental implants must be placed in the maxillary or mandible to replace lost teeth, first decisions that must be taken are about the size of the implants to choose and the protocols of implantation adopted to ensure the primary stability. According to the position where the implants are placed, they have a specific design. The main design parameters are diameter, length, shape and surface. Currently, the implants can vary in diameter from narrow, regular to wide (2). Also, the length of the implants is categorized as short, medium and long (2). Implants have reached the most frequent use in case of missing teeth as a single-tooth replacement, an implant is freestanding, not attached to adjacent teeth, and therefore, much easier to be cleaned. For patients who have lost multiple teeth, implants can provide support for a stable, healthy, and esthetic dentition that is not removable. Perhaps the most important aspect of implants is that they can restore quality of life (3).

The choice for a specific design always intends to reach the primary stability of the implant. The lack of primary stability can induce the formation of a soft tissue in the vicinity of implant. The implant mobility after surgery is the evident signal of failure caused by the formation of the fibrous tissue instead of the required osseointegration process. The stability depends on bone quality and implant design. The thickness of the cortical bone has a stronger influence for the primary stability and the length of the implants has weaker influence: the reason is that the cortical bone can provide a better stability instead the cancellous bone (4).

Wide diameter seems to present a great stability because it is evolved by cortical bone in a greater

Key words: fixtures, implant, fixture, bone, rehabilitation

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0393-974X (2020)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

area of contact. In addition, a value torque of 45 Ncm is usually required during wide implants placements. However, disadvantages are pointed due to the heating generated during the drilling of the bone for wide implants that can cause possible injury to the tissue. Especially for cortical bones the heating is higher (5). Instead, the narrower implants are indicated for residual ridge too narrow for regular implants and also for reduced width interdentally space. However, the use of implants in the extremely resorbed crestal bone due to neoplasm (6-17), malformations (18-24) and periodontal diseases (25-34) are still subjects of discussion.

Since a new type of fixture (Tuff type, Noris Medical Ltd. Nesher, Israel) has been introduced recently in the global market, we analyses a series of two-pieces implants inserted in different sites of lower and upper jaw in order to evaluate their survival (i.e. total number of fixtures still in place at the end of the follow-up) and success rate (i.e. peri-implant bone resorption).

MATERIALS AND METHODS

Study design/sample

To address the research purpose, the investigators designed a retrospective cohort study. Subjects were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity; in addition, the patients had to agree to participate in a post-operative check-up program.

Variables

Several variables are investigated: demographic (age and gender), anatomic (upper or lower jaws, tooth site), implant (length, diameter) and prosthetic (Toronto bridge, crown or ball attachment to fix removable prosthesis) variables.

The predictors of outcome are the percentage of implants still in place at the end of the follow-up period (i.e. survival rate – SVR) and the peri-implant bone resorption. The latter is defined as implant success rate (SCR) and it is evaluated according to the absence of persisting peri-implant bone resorption greater than 1.5 mm during the first year of loading and 0.2 mm/years during the following years.

Data collection methods

Before surgery, radiographic examinations were done with the use of intra-oral radiographs and orthopantomographs. Peri-implant crestal bone levels were evaluated by the calibrated examination of intra-oral radiographs and orthopantomograph X-rays after surgery and at the end of the follow-up period. The measurements were carried out medially and distally to each implant, calculating the distance between the implant' neck and the most coronal point of contact between bone and implant. The bone level recorded just after the surgical insertion of the implant was the reference point for the following measurements. The measurement was rounded off to the nearest 0.1 mm. The radiographs were performed with a computer system By knowing dimensions of the implant, it was possible to establish the distance from the medial and distal edges of the implant platform to the point of bone-implant contact (expressed in tenths of a millimetre) by doing a proportion.

The difference between the implant-abutment junction and the bone crestal level was defined as the Implant Abutment Junction (IAJ) and calculated at the time of operation and at the end of the follow-up. The delta IAJ is the difference between the IAJ at the last check-up and the IAJ recorded just after the operation. Delta IAJ medians were stratified according to the variables of interest.

Surgical protocol

All patients underwent the same surgical protocol. An antimicrobial prophylaxis was administered with 1gAmoxycillin 500 mg twice daily for 5 days starting 1 hour before surgery. Local anesthesia was induced by infiltration with articaine/epinephrine and post-surgical analgesic treatment was performed with 600 mg Ibuprofen twice daily for 3 days. Oral hygiene instructions were provided.

Two-piece implants were inserted (Tuff type, Noris Medical Ltd. Nesher, Israel) with a flap elevation approach. The implant neck was positioned at the alveolar crest level. A second operation was then performed after four months to loading by means a provisional prosthesis. The final restoration was usually delivered within 8 weeks. All patients were included in a strict hygiene recall.

Data analysis

Pearson-chisquaretestwasusedtodetectthose variables statistically associated to SVR and SCR.

RESULTS

In the period June-December 2017 102 two-piece spiral implants (Tuff type, Noris Medical Ltd. Nesher, Israel) were inserted, 51 in females and 51 in males. The median age was 56 ± 8 (min-max 36-73 years). Forty-eight implants were inserted in upper jaw and 54 in mandible. Fixtures replaced 21 incisors, 15 cuspids, 48 premolars and 18 molars. Implant' length was 10 mm, 11.30, and 13 mm in 29, 43 and 30 cases, respectively. Implant' diameter was 3.3 mm, 3.75 mm and 4.2 mm in 47, 30 and 25 cases, respectively.

Thirty-six fixtures were placed in totally edentulous patient and 66 in partially edentulous subjects. There were 16 single crowns, 50 implants bearing two or greater bridges, 4 removable dentures and 32 supporting Toronto bridge. The overall mean follow-up was 11 ± 1 months. One implant was lost, survival rate (SVR) = 99.9%. Then peri-implant bone resorption (success rate, SCR) was used to investigate peri-implant bone stability. No implant have a crestal bone resorption greater than 1.5 mm in the first year follow up. No studied variable has an effect on clinical outcome.

DISCUSSION

Osseointegrationisknownas"aprocesswhereby a clinically asymptomatic rigid fixation of alloplastic materials is achieved and maintained in bone during functional loading" (3).

Theimplantsdesign,orelse,thelength,diameter, shape and surface are important to obtain a primary stability and osseointegration in the early stages of implantation. Considering the diameter tends to have a strong influence on the primary stability, since this parameter is crucial for the stabilization in the cortical bone. However, the surface rough implants also tend to present a strong behaviour to culminate in the osseointegration compared to the smooth ones (3).

Amongvariousfactorsinpredictingthesuccess of implant therapy, factors determined by the patient are the volume and density of available bone (3,4). The use of multiple implants in patients that needs total oral rehabilitation can manage different kind of bone and this fact needs to use different surgical procedures for proper management of bone. It becomes so mandatory a straight treatment planning and must be closely discussed with prosthodontic colleagues and patients. Several surgical techniques can be used for suitable choices of treatment considering bone block grafting, ridge splitting, sinus lifting, immediate implant placement, socket preservation, guided bone regeneration, short implant, temporary mini implant, titanium mesh tray, etc. Occasionally, major surgeries including microvascular surgery and orthognathic surgery may be needed for reconstruction and discrepant correction of the jaws prior implant placement (6-17).

Bone quantity and quality are determined by the localization and type of bone according to the criteria established by Lekholm and Zarb (35). The authors found significant differences in stability as a function of localization, with lower ISQ values for the maxillary versus mandibular sites, which were explained by the greater stability of implants in dense cortical bone than in trabecular bone.

Inthepresentreportonlyoneimplant(Tufftype, Noris Medical Ltd. Nesher, Israel) was lost with a survival rate of 99.9%. Then peri-implant bone resorption was used to investigate SCR but no variable statistically affected implant outcome.

In conclusion the studied implants are reliable devices for oral rehabilitation with a very high SCR and SVR.

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JOURNAL OF BIOLOGICAL REGULATORS & HOMEOSTATIC AGENTS Vol. 34, no. 1 (S2), 19-24 (2020)

OSTEOGENESIS: HOW CAN IT BE STIMULATED?

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Biophysical therapy can be performed using inductive, capacitive, mechanic or implanted devices. The mechanism of action of physical stimuli is at a membrane level where the activation of calcium channels determines the enhancement of cell proliferation and the production of growth factors. Biophysical therapy should be performed using devices and modalities described in the literature. The biophysical stimulation of osteogenesis is effective in the enhancement of the biology of fracture healing in presence of a correct orthopedic treatment in terms of good alignment and stabilization at the fracture site. The choice of which method must be used depends on the segment of bone that has to be treated, the type of fracture and if it is possible to apply the device on the skin. The presence of internal or external fixation devices is not a contraindication.

The bone behaves like a mechanical-electrical transducer so that the mechanical stimuli are transformed, by piezoelectric systems inside the bone, into electrical signals biologically active on bone formation (1-10).

Learning about the electrical properties of bone and on the comprehension that in a fracture callus as in general in the presence of osteogenesis there is an electronegative potential, orthopedic surgeons use the electrical stimulation to promote osteogenesis to treat those pathologies in which it has stopped or delayed.

The electrical stimulation of osteogenesis is a methodic in the field of the "clinical biophysic" which studies the employ in the clinical practice of physic energies applied to biological systems. Biophysical stimulation includes methods for applying an electromagnetic, electrical, mechanical-ultrasonic force on a skeletal site and is distinct from other chemical methods of induction of osteogenesis. The stimulus to the formation of new bone can be induced through different techniques a) faradic systems in which electrodes are applied on the bone segment to be treated, b) electric fields directly on the bone of the capacitive systems, c) ultrasound applied to the bone, d) pulsed electromagnetic fields directly on the bone.

Since non-union can be happen also in dental and maxillofacial surgery after oncological resection (11-22), orthognatic surgery (23-29) and in infected wound we shortly reviewed main points of biophysical stimulation.

Mechanism of action

The different methods of biophysical stimulation of osteogenesis seem to have the as a target the cell membrane which absorb the stimuli and then redistribute them to the different cellular metabolic

Key words: therapy, osteogenesis, electromagnetic, fields, ultrasound

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0393-974X (2020)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

pathways. Particularly, PEMF can modify important physiological parameters of cells cultured in vitro like cell proliferation, signal transduction, transcription, synthesis and secretion of growth factors. PEMF can induce cell proliferation in mitogen stimulated lymphocytes and to improve IL-2 receptor expression and IL-2 utilization in lymphocytes from aged donors, which are characterized from defective production and utilization of this growth factor. PEMF exposure induces cell proliferation in human osteoblasts and chondrocytes cultured in vitro (1-10). Electromagnetic fields determine signal transduction by means of intracellular release of Ca2+ leading to an increase in cytosolic Ca2+ and an increase in activated cytoskeletal calmodulin. PEMF induce a dose-dependent increase in bone and cartilage differentiation and the up-regulation of mRNA expression of extracellular matrix molecules, proteoglycan, and type II collagen. The acceleration of chondrogenic differentiation is associated with increased expression of transforming growth factor b1 (TGFb1) mRNA and protein suggesting that the stimulation of TGFb1 may be a mechanism through which PEMF affect complex tissue behavior such as cell differentiation and through which the effects of PEMF may be amplified. PEMF are also postulated to act at a membrane level influencing signal transduction of several hormones or growth factors like PTH, IGFII and Adenosine A2a producing the amplification of their transmembrane receptors. PEMF exposure induces an upregulation of BMP-2,-4,-7 mRNAs depending on the duration of the exposure with a peak after 24 h. Sollazzo et al. (5) described an overall analysis about the effects of PEMF on human osteoblast-like cells in vitro, using microarray technology, demonstrating that PEMF enhance osteogenesis through the up and down regulation of several genes that regulate osteoblast proliferation and differentiation. More specifically, PEMF determined the up regulation of Runx2, which is the transcription factor essential for bone formation, through the over expression of HOXA 10. Moreover, PEMF determined the up regulation of AKT1 which maintain bone mass and turnover. PEMF exposure induces the overexpression of P2RX7, which determine the Ca2+ channels activation and calmodulin. PEMFs also modulate the expression of genes encoding for collagenous and noncollagenous extracellular matrix proteins (COLA2A Osteonectin) and cytoskeletal proteins (FN1 and VCL). PEMF reduce the expression of degradation processes of extracellular matrix both inducing the expression of TIMP1, an important metallo-peptidase inhibitor, and directly reducing metallo- peptidases expression (MMP-11). PEMFs seem to exert an anabolic effect on cells acting on it in different ways. PEMF are able to stimulate osteoblastic differentiation and proliferation. PEMF promote the production of extracellular matrix and mineralization while decreasing their degradation and absorption processes.

Therapeutic indications

The first therapeutic indications of biophysical methodic were in the treatment of delayed union and non-union (1-10). Although all the four biophysical methods were shown to be effective in treatment of pseudo-arthroses and delayed union, the most effective are PEMF. In any case every biophysical method cannot be useful if the orthopedic treatment is non correct and if there is a gap between the fragment of the fracture greater than half of the diameter of the skeletal segment to be treated. In studies performed by using inductive methods, that are less invasive and easier to handle than faradic systems, the healing rate obtained was between the 77% and the 90% of the cases. With capacitive systems were obtained good healing rate results (the 70% of the cases) but less when compared to PEMF. Both inductive and capacitive systems are particularly indicated in the presence of infected lesions and the infection does not interfere on the success of the therapy. Moreover, the inductive system was demonstrated to be more effective than surgery in the presence of infection. The biophysical treatment would be preferred in the first instance in respect of other surgical methods, when a delayed union or a nonunion must be treated in the presence of a good mechanical stabilization of the fracture with good reduction of the fragments.

PEMF and ultrasounds are indicated in the treatment of recent fractures in which the fracture site, the eventual exposition, the feature of the fracture, and the conditions of the patient (metabolic diseases, systemic diseases, therapies, style of life, smoking) are suggestive for difficulties in fracture healing (“at risk fractures”). At risk fractures are the 20% of all fractures and there are high probabilities of nonunion (25-50%). Therefore, the employ of biophysical stimulation appears indicated because of its particularly favorable cost/benefits ratio. Biophysical therapy can be employed when callus formation is scarce or absent bone. In randomized double-blind studies demonstrated that the employ of biophysical stimulation makes it possible to shorten the time needed for bone consolidation of about 25-30% and that it is possible to accelerate healing process of fractures that normally need over 4 months to heal. The Electrical stimulation also shortened the mean healing time in the presence of wide exposition of the fragments and extensive damage of soft tissues at the fracture site. The employ of a cast or of an external fixator didn’t interfere with PEMF action. The stimulation with capacitive fields was demonstrated to be effective for the treatment of stress fractures in the athletes with high healing rate (88%).

PEMF are also effective in the treatment of articular fractures were a damage of the cartilage is present. In these cases, PEMF allow to treat both cartilage and bone lesions. In fact, PEMF producing the amplification of transmembrane receptors of Adenosine A2a reduce the damage on the joint environment form inflammatory reaction limiting the catabolic effects of pro inflammatory cytokines on cartilage and producing an up regulation of proteoglycans synthesis. In experimental and clinical studies PEMF were demonstrated to favor the subchondral bone healing, to prevent subchondral sclerosis and degeneration of articular cartilage, thus limiting arthritis progression.

The tree methods of osteinduction described before are successfully employed to favour the fusion and integration of bone grafts in spinal fusions. In particular, the capacitive method was demonstrated to be effective in improving healing rate in patients undergone spinal fusion (65% placebo and 84% active device).

The inductive method is particularly effective in the conservative treatment of avascular necrosis of the femoral head (stages I and II of Ficat classification) significantly limiting the progression of the disease toward hip osteoarthritis thus reducing the need of total hip replacement.

To reach best results coming from the employ of the different biophysical methods, it is very important to strictly follow their clinical indications. Therefore, much attention must be paid to prescribe one method instead of another depending on the pathology to treat. In general, the methods in which short daily application times are needed require a prolonged treatment. For instance, in the case of delayed unions, the employ of ultrasound stimulation, that require 20 minutes of daily application time, can last one or three months after the treatment with inductive method that instead require daily application times over 6 h. For the inductive method, if treatment is performed with a daily application OF less of 5 h, the success rate is worse than the treatment done with the correct daily application of more than 6 h (60% *vs* 90%). Since the capacitive and ultrasound techniques require the direct applications of the device on the skin, it’s not possible to use them whenever the patients wear a cast.

The use of inductive systems is difficult in the treatment of the shoulder, where instead the capacitive technique is particularly indicated in the presence of an internal fixation and is not a contraindication for the use of biophysical techniques. However, the presence of an internal fixation with a plate can act as a shielding for ultrasounds. In this case ultrasounds must be applied opposite to the plate. In the case of infection are recommended the inductive and capacitive methods while the use of faradic systems appears cannot be used. Osteogenesis stimulation may be considered a useful technique for periodontal disease and peri-implantitis treatment, and it has been increased a great interest in the last years both in basic (2-16) and advanced research (17-31).

DISCUSSION

The improvement of osteogenesis is important because of the wide clinical applications it may have. This is possible by employing the biophysical methods if the physician follows the correct indications, modality and application times. Much research is present in the current literature to comprehend the mechanism of action through which physical forces produce their biologic effect. PEMF and capacitive systems are known to act in different ways on cell and tissues. A more detailed knowledge of the mechanism of action of these methods permitted the extension of their clinical applications. PEMF are an important example of that. In fact, they were originally employed in the treatment of delayed union and non-union, but since their chondro-protective properties were demonstrated, PEMF are successfully used also in the treatment of cartilage pathologies.

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JOURNAL OF BIOLOGICAL REGULATORS & HOMEOSTATIC AGENTS Vol. 34, no. 1 (S2), 25-29 (2020)

RETROSPECTIVE STUDY ON FORTY-EIGHT FIXTURES INSERTED IN UPPER JAW

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It is generally accepted that maxilla has a less bone quality than mandible and this fact makes implant rehabilitation more complex. Recently a new type of spiral implants has been introduced in the global market. Since few reports are available a retrospective study was performed. A total of 48 two-piece spiral implants were inserted, 21in female and 27 in males. The median age was 58 ± 8. Implants replaced 10 incisors, 9 cuspids, 25 premolars and 4 molars. Implant' length was 10 mm, 11.5 mm and 13 mm in 13, 24, and 11 cases, respectively. Implant' diameter was 3.3 mm, 3.75 mm and 4.2 mm in 25, 17 and 6 cases, respectively. One implant was lost, survival rate (SVR) = 97.91%. Then peri-implant bone resorption was used to investigate success rate (SCR). The mean bone resorption was 0.3 mm after an average period of 1 year follow up. In conclusion the implants studied are reliable devices for oral rehabilitation with a very high SCR and SVR.

The rehabilitation of the edentulous maxilla is a relatively common clinical problem. Missing dentition can be replaced by dentures (not appreciated by the patient because of their instability, discomfort and negative psychological impact), or implant-supported prosthesis, which could be the ideal solutions, although the lack of sufficient bone volume is a common problem (1-2). So, although dental implants have been accepted as a viable treatment option for completely and partially edentulous patients, the bone heights between 10 and 12 mm are considered the minimal amount of bone required to place implants of sufficient length to guarantee a good prognosis.

In the posterior maxilla, the proximity of the maxillary sinus and insufficient quality and quantity of alveolar bone to achieve implants favorable anchorage may create problems for implant rehabilitation and thus zygomatic implants have been recently proposed as a successful solution.

In conventional implantology, several solutions have been described to accomplish implant placement in these sites, such as sinus lift or the use of short/tilted implants.

Here we analyses a series of two-pieces spiral implants (Tuff type, Noris Medical Ltd. Nesher, Israel) in order to evaluate their survival (i.e. total number of fixtures still in place at the end of the follow-up) and success rate (i.e. peri-implant bone resorption).

MATERIALS AND METHODS

Study design/sample

To address the research purpose, the investigators designed a retrospective cohort study. The study population was composed of patients admitted at the private practice for evaluation and implant treatment

Key words: implant, fixture, bone, loading, spiral

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0393-974X (2020)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

between June and December 2017.

Subjects were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity; in addition, the patients had to agree to participate in a post-operative check-up program.

The exclusion criteria were as follows: bruxists, consumption of alcohol higher than 2 glasses of wine per day, localized radiation therapy of the oral cavity, antitumor chemotherapy, liver, blood and kidney diseases, immunosupressed patients, patients taking corticosteroids, pregnant women, inflammatory and autoimmune diseases of the oral cavity.

Variables

Several variables are investigated: demographic (age and gender), anatomic (tooth site, jaws), implant (length and diameter), and prosthetic (Toronto bridge, crown or ball attachment to fix removable prosthesis) variables.

The predictor of outcome is the percentage of implants still in place at the end of the follow-up period (i.e. survival rate - SVR) and the peri-implant bone resorption. The latter is defined as implant success rate (SCR) and it is evaluated according to the absence of persisting peri-implant bone resorption greater than 1.5 mm during the first year of loading and 0.2 mm/years during the following years.

Data collection methods

Before surgery, radiographic examinations were done with the use of intra-oral radiographs and orthopantomographs.

Peri-implant crestal bone levels were evaluated by the calibrated examination of intra-oral radiographs and orthopantomograph x-rays after surgery and at the end of the follow-up period. The measurements were carried out medially and distally to each implant, calculating the distance between the implants' neck and the most coronal point of contact between the bone and the implant. The bone level recorded just after the surgical insertion of the implant was the reference point for the following measurements. The measurement was rounded off to the nearest 0.1 mm. The radiographs were performed with a computer system as described elsewhere (1).

The difference between the implant-abutment junction and the bone crestal level was defined as the Implant Abutment Junction (IAJ) and calculated at the time of operation and at the end of the follow-up. The delta IAJ is the difference between the IAJ at the last check-up and the IAJ recorded just after the operation.

Surgical protocol

All patients underwent the same surgical protocol. An antimicrobial prophylaxis was administered with 1g Amoxycillin 500 mg twice daily for 5 days starting 1 hour before surgery. Local anesthesia was induced by infiltration with articaine/epinephrine and post-surgical analgesic treatment was performed with 600 mg Ibuprofen twice daily for 3 days. Oral hygiene instructions were provided.

Two-piece implants were inserted (Tuff type, Noris Medical Ltd. Nesher, Israel). The implant neck was positioned at the alveolar crest level. A second operation was then performed after four months to loading by means a provisional prosthesis. The final restoration was usually delivered within 8 weeks. All patients were included in a strict hygiene recall.

Data analysis

Pearson-chi square test was used to detect those variables statistically associated to SVR and SCR.

RESULTS

A total of 48 two-piece spiral implants were inserted, 21in female and 27 in males. The median age was 58 ± 8. Implants replaced 10 incisors, 9 cuspids, 25 premolars and 4 molars. Implant length was 10 mm, 11,5 mm and 13 mm in 13, 24, and 11 cases, respectively. Implant diameter was 3.3 mm, 3.75 mm and 4.2 mm in 25, 17, 6 cases, respectively. One implant was lost, survival rate (SVR) = 97.91%. Then peri-implant bone resorption was used to investigate success rate (SCR). The mean bone resorption was 0.3 mm after an average period of 1-year follow-up.

Statistical analysis performed by detecting bone resorption around implants did not demonstrated any variables which may have negative effect on outcome.

DISCUSSION

The anatomic and morphologic structure of the maxilla and the reduced bone volume caused by a high degree of resorption are critical in implant long-term success, indeed maxillary implants are generally loss successful than those in the mandible (3).

This is especially true if one considers very patients with co-morbidities such as post-oncological reconstruction (4-15), congenital malformation (16-22) or advanced periodontal diseases (23-32) with may negatively affect implant outcome.

In addition, various studied demonstrated that bone contacts differ when different titanium implant surface are used; significant advantages exist for roughened titanium surface implants in comparison to smoother titanium implant surfaces. Different implant manufactures have sought to enhance their surface topography and coatings; for example Institute Straumann manufactures implants with an SLA (sandblasted, large-grit, acid-etched) implant surface, while the Swiss Plus System has self-tapping apical threads and a microtextured surface on the intraosseous portion of the implant body (33).

Fixed prostheses in the maxilla are more successful than removable dentures. Prospective long-term studies presented by Fisher et al. show implant survival rates ranging from 95.5% to 97.9% where evaluating fixed full-arch bridges in the maxilla (34). Gallucci et al. (35) also affirmed that fixed implant prostheses in the edentulous maxilla are a scientifically validated treatment option.

In the present report only one implant was lost, survival rate = 97.91%. Peri-implant bone resorption was used to investigate the clinical success rate (SCR) among the remaining 47 implants. None has a crestal bone resorption greater than 1.5 mm so no variables can be associated to a potentially worse implant prognosis.

In conclusion the studied implants (Tuff type, Noris Medical Ltd. Nesher, Israel) are reliable devices for oral rehabilitation with a very high SCR and SVR.

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IMPLANT SUFACE ACTIVATES FIBROBLASTS: AN IN VITRO STUDY

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Titanium (Ti) is that the most generally used material for dental, orthopedic and maxillofacial purposes thanks to its excellent biocompatibility and mechanical properties. Several data suggest that prosthesis anchorage to bone and soft tissue are often modulated by surface characteristics. Fibroblasts are the soft tissues cells concerned in producing extracellular matrix and collagen and their tight connection to implant neck is of paramount importance in preventing peri-implant infection. The aim of this work is to grow Human Fibroblast (HFb) for seven days in wells containing (or not) dental implants. The expression levels of some adhesion and traction-resistance related genes (COL11A1, COL2A1, COL9A1, DSP, ELN, HAS1, and TFRC) were analyzed using Polymerase Chain Reaction. Our results demonstrated that several genes encoding for extracellular matrix proteins are activated so giving more insight to the comprehension of the mechanism of cell to surface adhesion.

Titanium (Ti) is the most widely used material in implantology for dental, orthopedic and maxillofacial purposes due to their excellent biocompatibility and mechanical properties (1-21). In implantology a considerable number of implants failed due to the unsuccessful integration of the material with the surrounding tissue. The main causes of this bad outcome are inflammation, loss of supporting bone and soft tissue recession (22-31).

Cell colonization of biomaterials is characterized by the modification of cytoskeleton followed by activation of genes related to proliferation, migration and differentiation.

The surface-design enhanced tissue attachment, fibroblast proliferation and the formation of connective tissue fibers perpendicular to the implant (24, 25), with the formation of a biological seal that prevented bacterial colonization.

Different biocompatible materials are currently employed for implants. Among these, Ti is considered a "gold standard" because of its biocompatibility and good corrosion resistance (1,2). Recently, a new type of implant (Noris Medical Ltd., Nesher, Israel) with a spiral form has been produced. The aim of this work is to verify the effect of titanium layer using Human Fibroblast (HFb) culture. The expression levels of some adhesion and traction-resistance related genes (COL11A1, COL2A1, COL9A1, DSP, ELN, HAS1, TFRC) were analyzed using Polymerase Chain Reaction.

MATERIALS AND METHODS

Titanium implants

Four implants (Tuff type, Noris Medical Ltd. Nesher, Israel) were placed in wells.

Key words: titanium, implants, fibroblast, gene, expression

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0393-974X (2020)

Cells Culture

For the investigation, HFb at the second passage were seeded on wells (with or without implants) The medium was changed three times a week and the cells were maintained in a humified atmosphere of 5% CO2 at 37°C. Cells were trypsinized and lysed for RNA extraction, after 7 days of treatment.

RNA processing and Real Time PCR

Reverse transcription to cDNA was performed directly from cultured cell lysate using the TaqMAn Gene Expression Cells-to-Ct Kit (Ambion Inc., Austin, TX, USA) following manufacturer's instructions. Briefly, cultured cells were lysed with lysis buffer and RNA released in this solution. Cell lysate were reverse transcribed to cDNA using the RT Enzyme Mix and appropriate RT buffer (Ambion Inc., Austin, TX, USA).

Finally, the cDNA was amplified by real-time PCR using Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and the specific assay designed for the investigated genes.

Expression was quantified using real time PCR. The gene expression levels were normalized to the expression of the housekeeping gene TFRC and were expressed as fold changes relative to the expression of the untreated HFb.

Forward and reverse primers for the selected genes were designed using primer express software (Applied Biosystems, Foster City, CA, USA) and are listed in Table I.

Table I. *Primers sequences.*

Gene symbol	Primer sequence (5'>3')
COL11A1	Fw AGATGAGGCAAACATCGTTGA Rev ATCAGAATCCCTGCCGTCTA
COL2A1	Fw GCGACGACATAATCTGTGA Rev GTCCTTTGGGTCCTACAATA
COL9A1	Fw GTAACAGTGAAGGGGTCGTGA Rev TTGGCCAATCCTGATCTTTG
DSP	Fw ATGACCTGAGGAGAGGACGAA RevAGGCTCTCTCTTTCCTGTACCAC
ELN	Fw CTAAATACGGTGCTGCTGGC Rev CATGGGATGGGGTTACAAAG
HAS1	Fw CTCGGAGATTCGGTGGACTA Rev CGCTGATGCAGGATACACAG
TFRC	Fw CGCTGGTCAGTTCGTGATTA Rev GCATTCCCGAAATCTGTTGT

Real Time PCR

All PCR reactions were performed in a 20 µl volume using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). Each reaction contained 10 µl 2X Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM concentration of each primer, and cDNA. All experiments were performed including non-template controls to exclude reagents contamination. PCRs were performed with two biological replicates.

RESULTS

Activation of extracellular matrix proteins were evaluated by measuring the gene expression levels after 7 days of treatment. Empty well was the control surface to compare the gene expression of implant surface. Fig. 1 showed the differentially expressed genes in the implants respect to the control surface.

DISCUSSION

A close joint between implant and surrounding tissues is essential for the maintenance and success of a dental implant in longevity. This result depends on the growth and adhesion of the cells of the periodontium to the tissue-implant interface.

The aim of this study was to compare fibroblasts behavior cultured in wells containing dental implants compared with empty wells.

After 7 days of treatment the expression levels of genes COL2A1, COL9A1, COL11A1, ELN, DSP and HAS1 were measured by relative quantification method using PCR. COL2A1, COL9A1 and COL11A1 are involved in conferring resistance to compressive forces (32). After 7 days of treatment we observed the up-regulation of all three collagens in fibroblast cultivated respect to control.

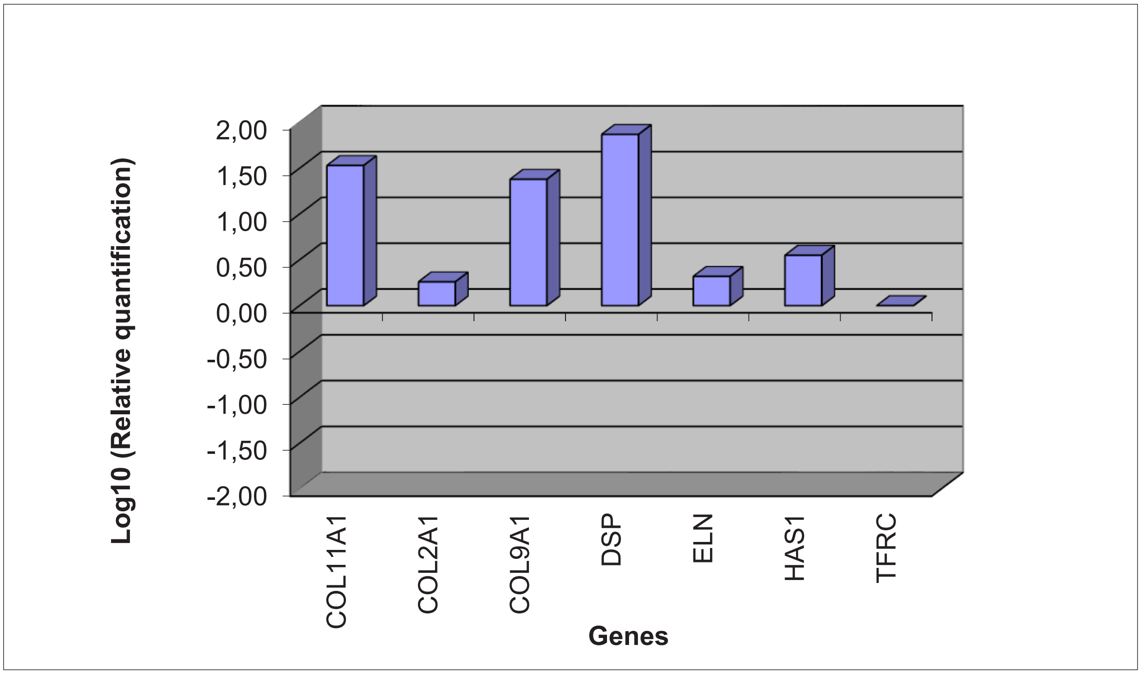

Fig. 1. *Gene expression in HFb.*

DSP encodes desmoplakine, an essential component of desmosomes that anchor intermediate filaments to desmosomal plaques. Its up-regulation suggests the involvement of this protein in cell-cell and cell-matrix adhesion. This gene was up-regulated.

ELN gene encodes for elastin, the major component of the amorphous phase in elastic fibers of human gingival. Elastic fibers are the extracellular matrix structures responsible for the properties of resilience and elastic recoil in all elastic tissues (33, 34). This elastic tissue that surrounds the dental implant neck, named peri-implant, is critical for a successful implant osseointegration as it forms a biological seal at the gingival site.

Another gene up-regulated was HAS1, the hyaluron synthase. This gene is essential in the synthesis of high molecular-weight hyaluronic acid. Hyaluronic acid is a major glycosaminoglycan in the periodontal ligament and has important roles in cell adhesion, migration and differentiation mediated by various hyaluronon-binding proteins and cell-surface receptors (34).

Our preliminary results demonstrate that implant surface (after 7 day of fibroblast culturing) promotes the production of protein involved in cell-cell and cell-matrix adhesion and in stress-resistance, required for a good outcome in dental implantology.

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