Periodontics

Stemness properties in high passage palatal and granulation tissue mesenchymal stem cells

Soanca Andrada*, Roman Alexandra*, Páll Emöke, Cenariu Mihai**, Rusu Darian***, Șoriță Olga******

*Department of Periodontology, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
**Department of Veterinary Reproduction, Obstetrics and Gynecology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
***Periodontology Department, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania
****Laboratory of Tumor Biology and Radiobiology, Prof. Dr. Ion Chiricuță Oncology Institute, Cluj-Napoca, Romania

Aim: The study investigated the persistence of stemness properties in high passage mesenchymal stem cells (MSCs) by analysing the surface antigen expression and their differentiation potential.
Methods: The study was approved by the Ethical Board of the university (359/13.10.2014) and was done accordingly to EU and national lows. MSCs were isolated from two oral sources: a palatal premolar region that was already used as a donor site for grafting and periodontal granulation tissue obtained after a pocket-reduction surgery. Re-entry derived cells (named reMSCs) and granulation periodontal tissue derived cells (named gtMSCs) were isolated using the explant method. Cells at 14th passage were used. Immunophenotype analysis identified specific surface antigens, with a FACS Canto II flow cytometer and the DIVA software. Trilineage Mesenchymal Differentiation potential was evaluated by culturing the cells in specific induction media. After 10 days of culture in the chondrogenic, osteogenic, and neurogenic differentiation medium, cells were fixed for immunocyto- and Alcian Blue staining, immunocyto- and Alizarin Red staining, and for immunocytostaining, respectively.
Results: The standard minimal criteria suggested by the International Society for Cellular Therapy such as adherence to plastic, specific surface antigen makeup and multipotent differentiation potential were fulfilled in the present experiment. Immunophenotype analysis revealed a similar expression pattern, but the level of antigen expression indicated some differences. Both cell lines showed positivity for CD105, CD73, CD90, CD44 and CD49f and a negative expression for CD34, CD45, HLA-DR and CD79a. At passage 14, both cell lines showed an intense expression of the positive markers, recording more than 99.3%, excepting CD90. After 10 days of exposure of reMSCs and gtMSCs to chondrogenic differentiation medium, Trilineage Mesenchymal Differentiation revealed the presence of rich mucopolysaccharides deposits in multi-cellular clusters by Alcian Blue staining. Immunocytostaining showed a weak positive expression of type II collagen. After 10 days of cultivation in selective neuronal medium, both MSC-derived cell lines expressed in different degrees neuronal antigens (glial fibrillar acidic protein, nestin and neurofilaments), suggesting their differentiation into neuronal progenitors, with a higher positivity for neurofilaments. Induction of osteogenic differentiation of MSCs was demonstrated by the presence of calcium deposits visualized by Alizarin red staining. Immunocytochemical staining for bone markers showed positivity for non-collagenic phosphoprotein ostepontin marked with FITC, which was more intensely expressed in ossification nodules derived from reMSCs in comparison with gtMSCs. A much weaker staining was observed for alkaline phosphatase.
Conclusions: The standard minimal criteria were fulfilled for high passage-MSCs as the antigen make-up and trilineage differentiation capacity demonstrated. It seems that palatal tissue regains its regenerative properties in terms of MSC presence in the reentry area.

The development of new resin composites for restoring tooth cervical lesions: physico-chemical testing

Condor Daniela*, Roman Alexandra*, Soancă Andrada*, Cioban Cosmin*, Moldovan Mărioara**

*Department of Periodontology, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
**Raluca Ripan Institute for Research in Chemistry, Babes-Bolyai University, Cluj-Napoca, Romania

Aim: Resin composite materials are frequently used to restore tooth loss located in the cervical part of the tooth (root-crown cervical lesions or RCCL) associated with gingival recessions and they could contact the surrounding gingival tissues when a combined restorative-surgical treatment of these lesions is required. The study aimed to develop a new, nano-hybrid resin composite material highly biocompatible to be used for restoring RCCL and to evaluate their water sorption and solubility- two important parameters of resin composites on mechanical properties, wear resistance, stability of polymeric network and biocompatibility of the restorations.
Methods: Materials. Three experimental composite resins were prepared in the laboratories of Raluca Ripan Institute for Research in Chemistry (ICCRR), based on a 2,2-bis(3-(2'-hydroxy-3'methacryloyl-oxypropoxy)phenyl)propane/ urethane dimetacrylate/ policaprolactone diol resin and a complex filler (78% wt) based on various combinations of containing hydroxylapatite-zirconium, silica (Degussa, Germany), and barium oxide glass filler. Three experimental variants were prepared: PM, P2S, P14M. Control dental composites were represented by two well-known marketed products recommended for the restoration of RCCL (G-aenial Anterior®, Enamel plus HRV®, named Ge and En). Standard disc-shaped composite specimens with standard dimensions (15 mm in diameter, 1 mm in thickness, n= 8 per composite type) were prepared. Measurement of water sorption and solubility was made in order to observe the stability of the experimental materials and to compare it with that of commercially available ones. The tests were done according to ISO 4049/2000 and ADA Specification No. 27 -2003. Statistical analysis was performed using a GraphPad Prism5.0 software
Results: The statistical analysis of water sorption data after 7 days revealed the best significant result for En, which was maintained over the entire observation period, followed by P14M and Ge that behaved similarly. At 14 and 90 days, no significant difference in sorption values was recorded for Ge and P2S, while P14M and PM were associated with the highest sorption parameters in comparison with the other materials. As for the water solubility test, again En had statistically the lowest solubility followed by Ge and P2S material, which behaved in the same manner all over the experiment. The solubility for P14M was not statistically different from that of Ge and P2S at 7 day moment but showed a worsening pattern at 14 and 90 days so that, at the final moment, it was not significantly different from the solubility value of the PM material. Sorption and solubility values in saliva showed an improved pattern for two experimental materials- PM and P2S, which was not significantly different statistically from that of commercial resin composites.
Conclusions: Two of the experimental resin composites behaved in the same manner as commercial materials all over the experiment. The weakest values recorded for one experimental resin composite may be due to the presence of policaprolactone, which may be degraded inducing an instability of the network and favorising the leakage of monomers and thus o possible hazardous effect on periodontal tissues.

Surgical-prosthetic treatment of soft tissue dehiscence around dental implants: a case report

Corana Matteo, Collivasone Dario, Zacconi Loris, Vezzoni Franco

Periodontal Unit, School of Dentistry, Department of Clinical-Surgical Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy

Aim: Peri-implant soft tissue dehiscence could be surgically corrected. In literature it was proposed to use subepithelial connective tissue grafts. The aim of this study is to describe an innovative surgical-prosthetic approach to correct soft tissue dehiscence around implant-supported single crowns.
Methods: A 23-year-old female patient presented with two implant-supported temporary crowns in the aesthetic area (1.2 and 2.2 positions). Both implants were characterized by labial soft tissue defects. As a consequence, the aesthetic outcome of the rehabilitation was compromised. The treatment was performed bilaterally and consisted of: abutment modification, subepithelial connective tissue graft excised from the maxillary tuberosity, progressive modification of a new temporary crown during the healing phase and, finally, placement of a lithium disilicate final restoration. The results of the treatment were evaluated in terms of probing pocket depth (PPD), modified Sulcus Bleeding Index (mBI), modified Plaque Index (mPI), percentage of soft tissue dehiscence coverage, height of keratinized tissue, marginal tissue thickness and Pink Esthetic Score (PES). Linear measurements were performed eighteen months after final restoration with an image

processing program (ImageJ, National Institutes of Health, USA). Patient-reported post-surgical pain was evaluated using a Visual Analogue Scale (VAS, 0 = no pain, 10 = very intense pain).
Results: Eighteen months after final restoration, the percentages of soft tissue dehiscence coverage were 62% (1.2 site) and 63% (2.2). Both height and thickness of marginal keratinized mucosa were increased, in the absence of inflammation (mBI=0), plaque accumulation (mPI=0) or pathological probing depth (PPD ≤ 3 mm). The PES of the right site was 4/14 before treatment and 11/14 after treatment; on the left the index increased from 7/14 to 12/14. The patient reported a low level of post-surgical pain (2-3 units in a 0 to 10 scale) and was really satisfied at the end of treatment. These results were stable four years after final restoration.
Conclusion: This surgical-prosthetic treatment was evaluated from a clinical and aesthetic point of view and the results were positive. In particular, the esthetic improvement was substantial. Moreover, the choice of the maxillary tuberosity as the graft donor site makes it possible to minimize patient post-surgical pain and discomfort. In conclusion, this method could be considered to correct soft tissue dehiscence around implant-supported single crowns in the aesthetic area and the PES is a useful index to evaluate the aesthetic outcome of peri-implant plastic surgery.

Periodontal plastic procedure: clinical efficacy of coronally advanced flap with or without connective tissue substitute for the treatment of multiple gingival recessions

Cutini Noemi, Antonelli Marco, Ferrari Marco, Grandini Simone, Discepoli Nicola

Department of Periodontology, University of Siena, Italy

Aim: The aim of the present study was to determine whether the addition of a xenogeneic collagen matrix, Geistlich Mucograft , to a coronally advanced flap (group B, Control) would improve the clinical outcome compared to a coronally advanced flap alone (group A, Test) for the treatment of multiple gingival recessions and for the reduction of hipersensitivity. The outcome variables considered were: Reduction of Gingival Recession (REC red), Increase of Keratinized tissue (KT), Complete Root Coverage (CRC). Furthermore both aesthetic index score (RES) and hipersensitivity were considered.
Methods: This study was a randomized, single-blind, parallel-group,superiority clinical trial. Sixteen subjects (N=16), presenting at least two adjacent Miller Class I or II multiple gingival recession, were enrolled in the study. After randomisation two groups were formed: group A (Control) all recessions were treated by coronally advanced flap (CAF); group B (Test) all recessions were treated by CAF plus the addition of a xenogeneic collagen matrix (XCM), Geistlich Mucograft, to coronally advanced flap (CAF+XCM). Surgeries were standardized and the same procedures / materials were employed to ensure the consistency of the study. The follow-up of REC Red, KT, CRC for individual patients were performed at 12 months. The follow-up for hypersensitivity was performed at 2 weeks, 1 month, 3 months and12 months. During this period subjects were monitored according to specific periodontal score at 1 week, 2 weeks , 1 month, 3 months , 6 months and 12 months after surgery. A single examiner, blinded to the treatment assignment, performed all measurements . A biostatistician, blinded to the treatment, performed the analysis of the data.
Results: A statistically significant difference was demonstrated between the two groups in terms of Rec Red, CRC, increase of KT, in favour of the group A (Test) The professional aesthetic results was higher in the test; after 1 year of follow-up, greater hypersensitivity reduction was observed in the test group.
Conclusion: Both clinical procedures can treat succesfully class I and II multiple adyacent recessions. With the limitation of the present clinical protocol, Test procedure (CAF+ XCM) seem to be more reliable in terms of CRC, RES and reduction of hipersensitivity at 1 year follow-up.

Influence of tissue thickness on root coverage: a randomized controlled clinical study

Danesi C., Clementini M., De Sanctis M.

Department of Periodontology, Dental School, Vita-Salute San Raffaele University, Milan

Aim: The coronally advanced flap (CAF) is a predictable procedure to treat gingival recessions. Several factors, as flap tension, the surgical positioning of the flap relative to the cemento-enamel junction and flap thickness, seem related to achieving complete root coverage (CRC). Some years ago a modification of CAF in flap elevation for single recessions was introduced. A split–full–split approach was used to elevate the flap in order to guarantee, at the level of the surgical papilla, anchorage and blood supply in the inter-proximal areas, to maintain the maximum soft tissue thickness above the root exposure, and to allow a coronal mobilization of the flap by means of a superficial incision eliminating lip muscle insertions residing in the thickness of the flap. In such a way, the portion of the flap residing over the previously exposed avascular root surface, including the periostium, may confer more thickness and better opportunity to achieve root coverage. In the current literature lots of studies utilizing CAF still describe the surgical technique as a complete partial-thickness flap,

obtaining in many instances good results in complete root coverage and stability over time. However, for such, it is not yet clear if the presence of the periosteum in part of the flap could play a role in achieving better percentages of CRC. The aim of this double blinded, controlled and randomized clinical trial is to compare the CAF performed either by a "split-full-split" and a "split" thickness flap elevation for the treatment of isolated-type gingival recessions in the upper jaw.
Methods: Thirty patients, presenting one (Miller Class I) gingival recessions were selected. Each patient was randomly assigned to the test group ("split-full-split" elevation) or the control group ("split" elevation). Clinical parameters recorded at baseline and at 6 months were: recession depth (R), probing depth (PD), clinical attachment level (CAL) and keratinized tissue (KT). Mean Root Coverage (MRC) and percentage of CRC were also calculated.
Results: Recession Reduction resulted in a significant CAL gain in both groups, whereas PD was not altered. In the test group, REC decreased from 2,4 mm. at baseline to 0,3 mm. at 6 months, corresponding to a mean root coverage (MRC) of 87,5%, whereas in the control group REC decreased from 2,5 mm. at baseline to 0,7 mm. at 6 months (MRC 73,6%). Complete root coverage was achieved at 6 months in 61% (test group) and 46% (control group). A significant KT increase was observed in test group.
Conclusion: Recession Reduction, MRC, CRC and KT increase were better for "split-full-split" group than "split" group. So the presence of the periosteum in part of the flap seems to play a role in achieving better recession reduction outcomes.

Periodontitis is independently associated with an increase in platelet count: a new potential link with cardiovascular diseases

Romandini Mario*,, De Tullio Ilaria*, Quaranta Ciro*, Ricci Angelo*, Romandini Pier Luigi***, Laforì Andreina****, Perfetti Giorgio***

*Department of Medical, Oral and Biotechnological Sciences, University of Chieti Pescara
*Department of Periodontology and Prosthodontics, "G. Eastman" Dental Hospital - Policlinico Umberto I
*Department of Oral and Maxillo-Facial Sciences, Sapienza University of Rome
****School of Dentistry, Catholic University of the Sacred Heart

Aim: Blood platelets have an important role in atherosclerosis and in the formation of thrombi. Basing on this rationale, many epidemiological studies have shown how an increase in platelet count (thrombocytosis) is associated with a subsequent development of fatal coronary heart disease (CHD). At the same time, it is well know how inflammatory and infective processes can results in an increase in the number of platelets, a phenomenon called "reactive thrombocytosis". So that, it is reasonable to hypothesize that also periodontitis, an infection-based inflammatory disease, could lead to an increase in circulating platelets. Our hypothesis is that periodontitis could cause a reactive thrombocytosis and this increase in number of platelets could potentially mediate the well documented association between periodontitis and CHD. However, no epidemiological nationally representative studies have examined the association between platelet count and periodontitis. The aim of this cross-sectional study is to evaluate, using KNHANES data, if there was in 2012 an association between periodontitis and platelet count in a representative sample of the South Korean population.
Methods: The platelet count was measured by the DC detection method (XE-2100D - Sysmex/Japan) in the participants blood samples collected after a 12-hour fasting period. The periodontal status was assessed by dentists using the Community Periodontal Index (CPI), which was dichotomized at participant level into periodontitis (CPI≥3 in at least one sextant) and no periodontitis (CPI≤2 in all sextants). All the statistical analyses were carried out considering the complex sampling design. A student t-test was initially used to compare platelet count values of periodontitis patients with the non-periodontitis ones. In order to examine the association between periodontitis and platelet count allowing for potential confounders, a multivariate linear regression analysis was applied controlling for age, sex, smoking and hypertension status, LDL and triglyceride blood levels, consumption frequency of coffee and red wine, educational level and BMI. A further analysis was finally performed categorizing the platelet count in quartiles, and carrying out a multivariate logistic regression analysis with the same covariates.
Results: A total of 5732 subjects over 19 years of age were examined, representative of 38,8 millions of adults. The participants affected by periodontitis were 1327 (20.2% weighted of the total). A statistically significant lower mean platelet count was found in periodontitis subjects (251.05 Thous/µL) compared to the non-periodontitis ones (256.10 Thous/µL) (MD:-5.046; 95% CI:-9.23 to -0.86; P=0.018). However, after controlling for confounders, the relationship was inverted. In fact, in the multivariate analysis, the periodontitis subjects showed to have a higher platelet count (MD:7.77 95% CI:1.14 to 14.40; P=0.022). Moreover, the odds ratio for the periodontitis subjects to be in the highest platelet count quartile was 1.41 (95% CI:1.014 to 1.969) when compared to the non-periodontitis ones.
Conclusions: Within the limitations of this study, in

this nationally representative sample periodontitis prevalence was independently associated with an increase in platelet count. Moreover, the presence of periodontitis increased the estimated odds to be in the highest quartile of platelet count of 41%. Further studies are necessary to verify if this increase in thrombocytes could represent a relevant mechanism linking periodontitis with CHD.

The personality traits, anxiety and depression, produce different periodontal outcomes: a 18-month follow-up study

Bertoldi Carlo*, Generali Luigi*, Ferrari Monica*, Lalla Michele, Venuta Marco***, Consolo Ugo***

*Department of Surgery, Medicine, Dentistry and Morphological Sciences with Transplant Surgery, Oncology and Regenerative Medicine Relevance – School of Dentistry -University of Modena and Reggio Emilia, Modena, Italy.
**Department of Economy "Marco Biagi"- University of Modena and Reggio Emilia, Modena, Italy
***Department of Diagnostics, Clinical and Public Health - University of Modena and Reggio Emilia, Modena, Italy

Aim: Psychological attitude, depression, anxiety and oral hygiene on oral health have been recorded and correlated in subjects with untreated periodontal conditions.
Methods: Periodontal data of forty systemically healthy-patients, affected by moderate periodontitis, were collected at baseline and 18 months later. The dental belief (DBQ) was self-administered to patients: a health-value scale (HV); two adherence-intent scales (AI1, AI2), were scored separately. The psychological variables were assessed by questionnaires administered by one expert operator. Personality traits were grouped into cluster including all the personality traits (cluster-A: paranoid, schizoid, schizotypal; cluster-B: borderline, antisocial, narcissistic, histrionic; cluster-C: avoidant, dependent, obsessive-compulsive) anxiety and depression were also assessed. Patients were instructed to oral hygiene measures, treated with cause-related therapy and with adjunctive surgical therapy, when needed. Data were expressed as mean ± standard deviation (m±SD). The comparisons were performed by nonparametric tests, Spearman correlation coefficients between variables were calculated and the relationships between the dependent variables and the independent ones were examined through the standard multiple regression models. The alpha Cronbach reliability coefficient for each HV, AI1 and AI2 scales was calculated by the item analysis procedure of Stata. The level of significance of the applied tests was the standard value P=0.05. Statistical analysis was performed by Stata, version 14.00.
Results: Cluster-A and B showed a consistent tendency for reduced levels of oral hygiene (increased full-mouth-plaque-score -FMPS), independently by therapy and supportive periodontal therapy scheme applied. This effect appeared particularly enhanced in correlation with poorly compliant attitude and unfavorable baseline dental indices. On the contrary, cluster-C seemed to be linked to clinically better indices, particularly in terms of full-mouth-bleeding-score (FMBS) and pocket depth. Anxiety resulted directly correlated with depression, cluster-B and C, but inversely with FMPS and the patient's requirement for professional oral-care. Additionally, depression was directly correlated with FMBS.
Conclusions: Anxiety and depression, both affective disturbances, have a reciprocal link, and opposite effect on plaque and bleeding score. Personality traits (stable throughout life) play a significant role in periodontal condition and therapy outcomes that are not entirely ascribable to the lack of observance of oral hygiene rules, since study design and statistical analysis take in account also hygienic and behavior parameters. The knowledge of psychological traits of patients but also of anxiety and depression score, could greatly improve periodontal treatment outcomes.

Etiology and characteristics of halitosis in patients attending a halitosis centre in North Italy

Chun Ching Liu, Deli Federico, Mohamed Fahad Zafar, Lorena Campanelli, Enrico Pigella, Federica Romano, Mario Aimetti

Department of Surgical Sciences, C.I.R. Dental School, University of Turin, Turin, Italy

Aim: As there are few studies in Europe describing the characteristics of breath malodour for large groups of patients, the aim of the present retrospective study was to analyse the etiology of halitosis among patients attending a specialized centre in North Italy and to relate the volatile sulphur compounds (VSC) to the cause of halitosis.
Methods: The records of 504 consecutive patients (227 men, 277 women), aged between 16 and 90 years, who complained of bad breath and visited the outpatient clinic for breath malodour (C.I.R. Dental School, Periodontology Department, University of Turin) from January 2010 to December 2015 were reviewed. The characteristics of halitosis were explored by considering demographic and clinical data based on organoleptic testing and on the levels of volatile sulphur compounds (VSC) quantified with a portable gas chromatograph. Data on self-perception of

halitosis were also gathered from a self-administered structured questionnaire.
Results: The majority of patients (86.1%) had complaints of bad breath from 1-5 years. Approximately half of the patients (53.0%) reported that their breath interfered with their family life and more than one-third (37.3%) was concerned with activities at the workplace or social life in the last month. Over 40% of the patients used some masking products to alleviate breath malodour. Based on the organoleptic test, the prevalence of breath malodour was 95.8%. Only in 21 patients no objective or questionable odour could be found (psychogenic halitosis). In 90.7% of the cases the halitosis had an intra-oral origin. Periodontitis and gingivitis were determined as the only cause of bad breath in 33.9% of subjects and in combination with tongue coating in 55.2%. A small portion of the patients (1.6%) had tongue coating as the only cause of bad breath. In about 1.4% of the cases, halitosis originated from the ear, nose and throat (ENT) region, with tonsillitis and sinusitis as the most frequent cause. Among the extra-oral causes, in approximately 0.8% of the patients a gastro-intestinal pathology was found. VSC concentrations were lower in the psychogenic halitosis group, whereas no statistically significant differences were detected when comparing intra-oral and extra-oral halitosis subjects except for dimethyl sulfide that was detected in higher concentration in ENT and extra-oral halitosis patients. A significant correlation was observed between organoleptic scores, VSC, number of sites with probing depth ≥ 6 mm, and tongue coating.
Conclusion: The present data support findings from other authors that breath malodour is mainly of oral origin, and emphasizes the central role of oral health professionals in halitosis diagnosis and treatment. However, the role of tongue coating as only cause of oral malodour may be questioned in the Italian population we studied.

Aptamer-enriched hydrogel promotes new bone deposition in a rat periodontal fenestration model

Parisi L., Rivara F., Costa C.A.*, Galli C., Lumetti S., Manfredi E., Palioto D.B.*, Macaluso G.M.

Dipartimento di Medicina e Chirurgia, Centro Universitario di Odontoiatria, Università di Parma, Parma, Italia
*Facultade de Odontologia de Ribeirao Preto, Universidade de Sao Paulo, Brasile

Aim: In recent years, tissue engineering scaffolds have been proposed for periodontal regeneration. In the past, our group has demonstrated the ability of aptamer-loaded biomaterials to promote selective protein adsorption and improved osteoblasts adhesion in vitro. Here, we propose the innovative use of a hyaluronic acid/polyethylene glycol-based hydrogel (tHA/PEGDA) enriched with anti-fibronectin aptamers to enhance periodontal regeneration in a rat model.
Methods: Periodontal fenestration defects were created on the right mandibular side of 45 4-month-old Wistar Kyoto rats. Briefly, an extra oral incision was performed in the mandibular base, the inferior ligament border was cut and the muscle separated from the bone. Subsequently, the defect was created to expose the roots of the last two lower molars. In the mean time, tHA/PEGDA hydrogels were prepared with or without anti-fibronectin aptamers: 15 rats received 20µl of aptamer-enriched gel, 15 rats received 20µl of control gel while 15 rats were used as spontaneous healing control group. Euthanasia was performed 5, 15 and 30 days after surgeries and tissue regeneration was evaluated through microCT analysis.
Results: MicroCT analysis showed that bone volume (BV/TV) was significantly increased in tHA/PEGDA-filled defects by day 15 versus control ($p=0.0166$) and aptamer-enriched tHA/PEGDA hydrogel groups ($p=0.0389$). By day 30, BV/TV was still increased in defects treated with tHA/PEGDA versus control ($p=0.0139$), while defects with aptamer-enriched tHA/PEGDA presented the highest amount of BV/TV as compared to both control ($p<0.0001$) and tHA/PEGDA hydrogel groups ($p=0.0004$). MicroCT analysis furthermore revealed a decrease in porosity in tHA/PEGDA-treated defects versus control by day 15 ($p=0.0158$). By day 30 both defects treated with tHA/PEGDA and aptamer-enriched tHA/PEGDA showed a different porosity between them ($p=0.0117$) and a gradual decreased of porosity, as compared to control group (control VS tHA/PEGDA $p=0.0073$; control VS aptamer-enriched tHA/PEGDA $p<0.0001$).
Conclusions: Our data demonstrated for the first time the suitability of the aptamer loaded hydrogel as a periodontal tissue engineering scaffold in an animal model. Moreover, we demonstrated enhance tissue healing, with higher new bone deposition. Future analysis will aim to investigate the cell phenotypes involved in tissue regeneration and in loco gene expression.

Nutraceutics and periodontics: the effect of kiwi-fruit intake before and after periodontal therapy

Graziani Filippo*,, Discepoli Nicola*,**, Gennai Stefano*,**, Karapetsa Dimitra*,**, Lillo Luca*, Nisi Marco*, Petrini Morena*,****

*Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy
**Sub-Unit of Periodontology, Halitosis and Periodontal Medicine,

University Hospital of Pisa, Pisa, Italy

Aim: Periodontitis is an inflammatory disease that causes the irreversible destruction of supporting bone and ligament tissues of the teeth. It is the results of an excessive and imbalanced answer of the host defence toward bacterial stimulus. A large evidence highlighted the pathobiont role of bacteria that are able to promote pathology only when specific conditions are altered in the host. Indeed many influencers contributes to the development of periodontal disease, like genetic, systemic and local factors. Consequently focus should not only be on plaque control and removal of bacteria but also on improving host resistance through smoking abstention, stress reduction and a healthy diet. The importance of micronutrients has been recently extensively reviewed and concluded that for prevention and treatment of periodontitis daily nutrition should include sufficient antioxidants, vitamin D and calcium. Among vitamins, It is known that plasma vitamin C levels are inversely related to the severity of periodontitis and their deficiency are correlated with the development of necrotizing ulcerative gingivitis. Moreover literature has shown that periodontitis patients have a reduced intake and lower plasma levels of vitamin C compared to healthy controls. Good sources of vitamin C include peppers, strawberries, broccoli, brussels sprouts, oranges, grapefruits and kiwifruit. The aim of this study is to evaluate the effect of kiwi-fruit intake on systemic and periodontal health.
Methods: Two randomized groups were considered: cases consumed 2 kiwi-fruits daily and controls avoided to eat this fruit during the study. After the second month of this diet, all subjects were subjected to a session of full-mouth no-surgical periodontal therapy. Periodontal examination and blood collection, evaluating lipids profile and C-reactive protein, and vital signs were performed at baseline, after 2 and 5 months.
Results: At baseline both groups were characterized by similar periodontal and systemic values, but after 2 months of kiwi-fruit consumptions, cases were characterized by lower bleeding on probing, plaque and attachment loss, respect controls. After treatment, periodontal health clinically increased in both groups, however cases were characterized also by a statistically significant reduction of diastolic blood pressure and HDL level, respect controls.
Conclusions: The positive effect of nutraceutics on periodontal health has been confirmed: daily kiwifruit consumption determines a significant reduction of gingival inflammation in untreated periodontal disease.

The influence of periodontal disease on the development of diabetic complications

Graziani Filippo*,, Gennai Stefano*,**, Karapetsa Dimitra*,**, Lillo Luca*, Nisi Marco*, Petrini Morena*,****

*Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy
**Sub-Unit of Periodontology, Halitosis and Periodontal Medicine, University Hospital of Pisa, Pisa, Italy

Aim: An increasing evidence links the periodontal disease to inflammatory systemic disorders, like hypertension, diabetes and cerebro-cardiovascular conditions. The study of this relationship is very important, because according to the Global Report on Diabetes published in 2016 by the World Health Organization, about 50 % of people in the world suffer of periodontal disease and the global prevalence of diabetes increased from 4.7% in 1980 to 8.5% in 2014, in adult population. About 3.7 million of deaths worldwide in 2012 have been related to diabetic complications, like cardiovascular and other diseases and 43% of these occurred in people younger than 70 years old. Moreover literature has shown that diabetic patients have an increased risk to develop periodontitis, respect healthy population. The aim of this work is to analyse the effect of periodontal disease on the development of complications in diabetic patients respect periodontal healthy people.
Methods: A systematic literature review has been performed through hand and electronic search; in particular, PUBMED and EMBASE databases have been used to find articles published until January 2017.
Studies to be included had to be non-intervention, observational studies such as cohort, case-control (cases represented by periodontally-affected subjects and controls by non periodontally –affected subjects) or cross-sectional in design. In the selected studies, exposure had to be periodontal status (measures of inflammation, sings of disease such as pocketing and attachment level excluding tooth loss/edentulousims) of the included diabetic subjects and outcome one parameter related to diabetes such as measures of glucidic metabolism (glycated hemoglobin (Hb1Ac), fasting and 2-h 75 g plasma glucose (FBG) level and oral glucose tolerance test (OGT), incidence of new cases, diabetes-related complications. Only studies in English language were selected. Once completed the electronic and hand searching and after the elimination of papers present in duplicates, a total of 2891 studies were identified for inclusion in this review. After title, abstract and full-text analysis, a total of 12 studies were included.
Results: Literature analysis has shown that diabetic patients affected by periodontitis were characterized by higher incidence of diabetic complications, like retinopathy, ophthalmic disease, nephropathy

(macroalbuminuria, proteinuria and end-stage renal disease), neuropathic foot ulceration, various cardiovascular disease and death due to cardio-renal disease.
Conclusion: periodontal disease is associated to a higher risk of development diabetic complications.

Dental pulp stem cells in regenerative treatment of intrabony defects: a 12-month randomized controlled trial

Piccoli Gian Marco, Citterio Filippo, Del Buono Wilma, Giraudi Marta, Ferrarotti Francesco, Romano Federica, Aimetti Mario

Department of Surgical Sciences, C.I.R. Dental School, University of Turin, Turin, Italy

Aim: In the last years periodontal research has been pointed on tissue engineering to increase the regenerative potential of the injured tissues, in order to reduce complications and make the results more predictable. Stem cells are provided of unique abilities of proliferation and differentiation. They are addressed to tissue development during the embryogenesis and to tissue healing and homeostasis after the birth, when they are called postnatal or adult stem cells. Owing their accessibility and biological features dental pulp stem cells (DPSCs) hold great promise for regeneration in clinical applications. The present study aimed to explore the clinical and radiographic effectiveness of 3D biocomplexes combining commercially available collagen scaffold with autologous DPSCs micro-grafts in the treatment of non-contained intrabony defects in chronic periodontitis patients.
Methods: This trial was designed as a single-centre, randomized-controlled, parallel group study of 12 months duration. Twenty-seven generalized chronic periodontitis patients who had at least one residual non-contained periodontal intrabony defect after the completion of non-surgical therapy and one vital third molar requiring extraction were consecutively included in the study. Fourteen bony defects (3 maxillary and 11 mandibular) were treated with minimally invasive surgery technique (MIST) and DPSCs/collagen sponge biocomplex (test group), while 13 (7 maxillary and 6 mandibular) with MIST and collagen sponge alone (control group). The third molar was extracted and used as autologous DPSCs source. The pulp tissue was mechanically dissociated and passed through disposable 50µm filters in 1.2 ml of sterile physiologic solution to obtain a cellular suspension enriched in progenitor stem cells and endorsed onto a collagen sponge. Clinical and radiographic measurements were assessed at baseline, 6 and 12 months post-surgery.
Results: The healing phase was uneventful. Changes in clinical parameters over the 12-month period were significantly different between the two procedures ($p < 0.001$). The test group showed greater CAL gain and PD reduction at both re-evaluation assessments but not in REC changes. An overall PD reduction of 4.7 ± 1.3 mm and CAL gain of 4.4 ± 1.8 mm ($p < 0.001$) were observed at the end of the experimental period in the DPSC-treated group. In the control group PD values decreased on average by 3.0 ± 1.4 mm and CAL values by 2.6 ± 1.7 mm ($p < 0.001$). A greater radiographic bone fill was detected in the test compared with the control sites (3.4 ± 1.6 mm versus 1.7 ± 1.2 mm, $p = 0.005$) at 12-month follow-up examination.
Conclusion: The clinical and radiographic findings suggest that the application of autologous DPSCs may represent a very promising tool for the treatment of angular bone defects with unfavourable architecture. Based on the lack of histological analysis, a periodontal regeneration should be interpreted with caution.

The effect of some resin composites on palatal and granulation tissue mesenchymal stem cells

Roman Alexandra*, Páll Emöke*, Moldovan Mărioara*, Soanca Andrada*, Rusu Darian****, Șorițău Olga*****

*Department of Periodontology, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
**Department of Veterinary Reproduction, Obstetrics and Gynecology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
***Raluca Ripan Institute for Research in Chemistry, Babes-Bolyai University, Cluj-Napoca, Romania
****Periodontology Department, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania
*****Laboratory of Tumor Biology and Radiobiology, Prof. Dr. Ion Chiricuță Oncology Institute, Cluj-Napoca, Romania

Aim: As other dental materials used in the oral cavity, resin composites are not inert and could have hazardous effects on gingival cells due to eluted components. When placed for restoring root-crown cervical lesions during simultaneous reconstructive-root coverage interventions, freshly placed resin composites could hamper the healing of root surgical coverage. The present study aimed to evaluate the in vitro cytotoxic effects of five resin composites (two commercial and three experimental materials) on mesenchymal stem cells (MSCs) previously isolated from two oral sources.
Methods: Standardised disc-shaped specimens were prepared from three experimental composite resins (P14M, PM, P2S) produced by an autochthonous research institute and from two marketed products (G-aenial Anterior®, Enamel plus HRV®, named Ge and En). Palatal MSCs (obtained from a previous

palatal donor site) (reMSCs) and granulation tissue MSCs (gtMSCs), isolated and characterised in another experiment, at passage 14 were used. Alamar blue viability test investigated cell adhesion and viability. Cells were seeded onto the surface of composite discs at a concentration of 8x104 cells/ well. After 21 hours, 3 and 7 days of cultivation, Alamar Blue was added to each well. The fluorescence intensity was measured using a BioTek Synergy 2 plate reader. Cell membrane fluorescent labelling with PKH26 Red Fluorescent Cell Linker Kits was done. The cells were visualized by fluorescence microscopy with a Zeiss Axiovert D1 microscope, after 21 h, 3 and 7 days of cultivation onto composite discs. Statistical analysis. Two-way mixed ANOVA was used; our design included a within-subjects factor, Time and a between-subjects factor, Material type. Pairwise differences between material types were analyzed using one-way ANOVA, followed by Bonferroni post-hoc comparison. Statistical analysis was performed using a GraphPad Prism5.0 software.

Results: The statistical analysis of the data representing fluorescence unit count after Alamar Blue viability test revealed a significant 2-way interaction between Time and Material type ($p = .001$). For reMSC, data collected after 21h and 72h, the effect of Material type was not significant. For data collected on day 7, a significant main effect of Material type was found, Pairwise comparisons were found to be statistically significant for Ge vs.P2S, Ge vs.PM and Ge vs.P14M ($p < 0.05$), all other comparisons not being significant. Regarding statistical results of cell count for gtMSCs, a significant main effect of Material type and a significant pairwise comparison between Ge vs.P2S ($p < 0.05$) were found only for day 7. Global statistical tests and post-hoc comparisons for data collected at 21h and 72h were not significant. Cell membrane fluorescent labelling. The PKH analysis showed an obvious development of reMSCs during the entire observation period; a more discrete presence of gtMSCs was reccorded.

Conclusions: No signs of cytotoxicity of resin composites on MSCs were highlighted. No statistical differences in MSC development were observed excepting Ge that seemed more biologically attractive than all the other experimental materials after 7 days and P2S that seemed to be more biocompatible than Ge after 72 h.

Effect of a connective tissue graft in combination with single flap approach in the regenerative treatment of intraosseous defects

Simonelli Anna*, Farina Roberto*, Minenna Luigi*, Rasperini Giulio, Trombelli Leonardo***

*Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara, Ferrara, Italy
**Department of Biomedical, Surgical, and Dental Sciences, University of Milan, Milan, Italy

Aim: Post-surgery increase in gingival recession (REC) is a highly prevalent, undesired side effect of the surgical treatment of periodontal intraosseous defects, partly depending on flap design. In this context, we proposed the Single Flap Approach (SFA), a simplified surgical procedure for the treatment of intraosseous defects. A recent study has demonstrated that buccal SFA, when used to access a periodontal intraosseous defect associated with a buccal bone dehiscence, results in a post-surgery buccal REC (bREC) and that the severity of such REC is directly correlated with the severity of the bone dehiscence. In the attempt to limit the post-surgery increase in buccal gingival recession, the effect of a connective tissue graft (CTG) when combined with a buccal SFA in the regenerative treatment of intraosseous defects was evaluated.

Methods: Data related to 30 patients with a periodontal intraosseous defect treated with buccal SFA with (SFA+CTG group; n= 15) or without (SFA group; n= 15) the placement of a CTG were retrospectively derived at three clinical centers. In both treatments groups, buccal SFA was combined with a bovine-derived xenograft and EMD. bREC and probing parameters were assessed immediately before surgery and 6 months after surgery. The 6-month change in bREC was considered as the primary outcome variable of the study.

Results: In addition to a significant attachment gain and probing depth reduction, the adjunctive use of a CTG to a buccal SFA in the regenerative treatment of periodontal intraosseous defects associated with a buccal bone dehiscence resulted in a limited post-surgery bREC, a lower prevalence of defects with a clinically-detectable apical displacement of the gingival margin and an increase in gingival width and thickness.

Conclusions: In addition to a significant Clinical Attachment Level (CAL) gain and Probing Depth (PD) reduction, the adjunctive use of a CTG to a buccal SFA in the regenerative treatment of periodontal intraosseous defects associated with a buccal bone dehiscence resulted in a limited post-surgery bREC, a lower prevalence of defects with a clinically-detectable apical displacement of the gingival margin and an increase in gingival width and thickness.

The key periodontal pathogens in adults affected by chronic periodontitis with and without type 2 diabetes mellitus: a comparative study

Valeriani Leoluca, Gatto Maria Rosaria, Checchi Luigi, Montevecchi Marco

Reparto di Parodontologia ed Implantologia, Dipartimento di Scienze Biomediche e Neuromotorie, Alma Mater Studiorum, Università degli Studi di Bologna, Bologna, Italia

Aim: The aim of this study was to compare the prevalence and bacterial load of six main periodontal pathogens among chronic periodontitis patients with or without type 2 diabetes mellitus.
Methods: After selecting 20 diabetic patients (test group), a retrospective trial has been developed with a group ratio 1:1, using as matching variable the extension and severity of periodontal damage. Data were obtained from the Periodontal and Implantology Division database at Bologna University. Inclusion criteria were: diagnosis of chronic periodontitis, diagnosis of type 2 diabetes mellitus (only for the test group), presence of at least 12 teeth, age higher than 18 years, and caucasian race. Patients were excluded if they were pregnant or lactating, required systemic antibiotics within 3 months prior to the microbiological testing or anti-inflammatory therapy in the month before the visit, if they were suffering from any other systemic disease except diabetes mellitus (arthritis, ulcerative colitis, Crohn's disease, osteoporosis or osteopenia, HIV infection, hematologic diseases, neoplastic diseases, cardiovascular diseases or other pathologies that could potentially interfere with the periodontitis and/or with diabetes), if they suffered from mental disorders and/or had received periodontal treatment in the 6 months before the microbial test. The microbiological data were recorded during the first visit and obtained by means of a quantitative real¬time¬PCR. The bacterial load of each species, the total bacterial load of the single periodontal site, and the percentage of pathogens compared to the total load were analyzed. The bacterial pathogens examined were: Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, Fusobacterium nucleatum and Tannerella forsythia. The study protocol was previously approved by the Bologna-Imola Ethics Committee (Reference Number: EC 16044).
Results: The 2 groups resulted balanced in terms of demographic and clinical parameters, except for suppuration. The diabetic patients were 9 Female and 11 Male with a mean age of 56.5 ± 9 years while non¬diabetic patients (10 female, 10 male) had a mean age of 49.5 ± 14 years. In the microbiological test sites (4 for each patient) the mean probing pocket depth was 6.33 ± 1.62 mm in diabetic patients and 6.42 ± 1.77 mm in non¬diabetic patients, while sites with suppuration were 5 in the test group and 25 in the other. All diabetics were subjected to a metabolic control regime (metformin, insulin and/or diet control). The 65% of them suffered of diabetes for less than 15 years. Results show that diabetic patients had significantly greater amount of total bacterial load, red complex (Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia) and Fusobacterium nucleatum ($p<0,05$). Comparing to the total bacterial load, only Tannerella forsythia maintained its prevalence in diabetic patients ($p=0,0001$).
Conclusion: This retrospective study supports the hypothesis that microbiological differences exist between periodontal subjects affected and not by diabetes mellitus. Further investigations on broader samples and larger bacterial range are recommended.

Oral health of pregnant women: clinical assessment of the interlinkages with adverse pregnancy outcomes. A cross-sectional study

Gallenzi P., Patini R., Vasina M., Triestino A., A Giordano., Cordaro M.

Clinical Dentistry Institute, Università Cattolica del Sacro Cuore, Dean: Prof. Massimo Cordaro

Methods: The authors enrolled and analyzed a sample of 31 mothers (aged between 24 and 43 years) who had given birth at the Teaching Hospital "Agostino Gemelli" Foundation. All patients were subjected to an anamnestic questionnaire to obtain data on their lifestyle, state of health and oral hygiene habits. The patients were examinated in the Dental Clinic of the same hospital. The periodontal examination included the measurement of the amount of gingival recession (REC) and of the probing pocket depth (PPD) for each tooth excluding the third molars. Six sites were examined using a Williams probe. Bleeding on probing (BOP/BI) and plaque index (PI) were recorded in 4 sites (vestibular, mesial-buccal, disto-buccal, buccal) and expressed in percentages.
Results: The sample consists of 7 patients with preterm birth and 24 with fixed-term birth. The authors decided to classify the detected periodontal status in three categories: "healthy" (all sites with CAL less than 4 mm), "moderately affected" (at least one site with CAL between 4 mm and 6 mm) and "severely compromised" (at least one site with CAL greater than or equal to 6 mm). Data show important differences between the test and the control group. In the test group, 28.5% of patients revealed a seriously compromised periodontal status compared to 4.2% in the control group. In addition, the percentage of healthy patients in the control group was 37.5% against 14.2% of the test group. Bleeding on probing (BOP/BI) was 14.1% on average in women with pre-term birth compared to 7.1% of patients with fixed-term birth. Plaque index (PI) was 23.7% on average in the test group than 14.1% of the control group.
Conclusion: An association between periodontal

disease and pre-term birth was demonstrated. Consequently, the authors support American Academy of Periodontology(AAP)'s indications stated in 2004. According to the AAP, frequent dental visits and regular oral hygiene sessions in pregnant patients contribute in maintaining a good level of oral health, in preventing oro-dental diseases and in reducing the negative effects of oral infections during pregnancy. Periodontal status examination by dentists or dental hygienists in early pregnancy should be a routine medical intervention. Furthermore, pregnant women should undergo an intervention program to develope high oral hygiene standards during the whole gestational period. The authors agree on the need for extended, rigorous and homogeneous studies to clarify the association between periodontal disease and adverse pregnancy outcomes.

Full mouth erythritol power air polishing therapy (FM-EPAPT): a new way to make prophylaxis

Dalè Matteo

Università degli Studi di Brescia

Aim: The aim of this study was to compare FM-EPAPT with traditional ultrasonic debridement (USD) in terms of effectiveness in supragingival plaque removal, time required, patient's comfort, operator comfort, cleanliness and smoothness perception and experienced pain.
Methods: 14 systemically healthy young patients, both smokers and non-smokers, were selected. An informed consent was signed by all the enrolled subjects. The preliminary preparation consisted of a rinse with chlorhexidine 0,12% for 60 second, the placement of Optra-Gate® (Ivoclar Vivadent) and the application of the disclosing plaque agent (MIRA-2-TON®, 60 ml bottle, Hager Werken). The patients were therefore treated in split-mouth with two different treatments (USD and FM-EPAPT) randomly assigned to the quadrants 1-4 or 2-3. USD treatment consisted in:

- Calculus removal with a piezoceramic device (Air Flow Master Piezon® EMS Nyon, Switzerland) with a thin tip (PS® EMS, Nyon, Switzerland).
- Plaque and biofilm removal with a soft rubber cup (Pro Cup Soft Light Blue® Kerr, Bioggio, Switzerland) and polishing paste with a low RDA (Cleanic®, Kerr, Bioggio, Switzerland).

FM-EPAPT treatment consisted in:

- Decontamination of soft tissues with an air-polishing device (Air Flow Master Piezon® EMS Nyon, Switzerland) and Erythritol powder (Plus®, EMS, Nyon, Switzerland).
- Supragingival biofilm removal with an air-polishing device and Erythritol powder.
- Subgingival (up to 4 mm) biofilm removal with an air-polishing device and Erythritol powder.
- Removal of eventual calculus with a piezoceramic device (Air Flow Master Piezon® EMS Nyon, Switzerland) with a thin tip (PS® EMS, Nyon, Switzerland). At the end of both therapies, the disclosing agent was applied again to check the efficacy in biofilm removal. Time required for each treatment was registered. Vestibular and palatal/lingual photos of the frontal teeth (from 13 to 23 and from 43 to 33) were collected at baseline, after disclosing agent application, at the end of the therapy and after a second application of the disclosing agent. Also, all the patients were asked to fill an anonymous questionnaire scoring the experienced pain, the comfort and the sensation of smoothness and cleanliness on a scale from 0 to 5 for each side of the mouth. Same was asked to the operator with regards of the working comfort. The assessment of the efficacy in terms of biofilm removal was done by external unexperienced evaluator (a dental student) on the clinical post-treatment pictures. Basing on the residual disclosing agent on the teeth surfaces, a score from 0 to 3 was assigned to 4 tooth areas (mesio-vestibular, disto-vestibular, mesio-palatal, disto-palatal or mesio-vestibular, disto-vestibular, mesio-lingual, disto-lingual). Statistical analysis was performed using Linear Mixed Models (for quantitative variables) or Cumulative Link Mixed Models (for ordinal variables) with probit link.

Results: For probit model a positive effect estimate indicates an higher score for FM-EPAPT vs USD. All FM-EPAPT scores resulted significantly better than the ones obtained by USD, except for the smoothness and cleanliness perception (FM-EPAPT vs USD):

- Time spent -61.5" ($p=0.02$)
- Pain: -2.52 ($p = 0.0001$)
- Patient's comfort: 2.54 ($p < 0.0001$)
- Smoothness and cleanliness perception: 0.22 ($p =0.65$)
- Operator comfort: 1.55 ($p = 0.003$)
- Efficacy in biofilm removal: -0.63 ($p=0.0032$)

Conclusion: FM-EPAPT novel protocol seems to be able to achieve the same clinical results of the classical treatment with mechanical instruments and prophylaxis cups and pastes with a minor time consumption. Our choice to shift towards this new concept is led by the willing of making our patients and our operators as much comfortable as possible, in order to obtain a strong compliance and an environment that allows an easier oral healthcare provision.