Hyaluronic acid and its use in dentistry

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The purpose of this paper is to evaluate the role of hyaluronic acid in bio-revitalization (HABR) by testing several extracellular matrix biological parameters in cultured dermal fibroblasts. To this aim, fibroblastic expressed genes after exposition to three HABR medical devices were evaluated. Cells were seeded on a layer of three different medical devices containing 6.2, 10 and 20 mg/ml of HABR for 24 h. Real Time Polymerase Chain Reaction was performed to investigate gene expressions. Genes encoding HABR synthesis and degradation, Metalloproteinases 2 and 3 and Desmoplakin production as well as GDF6, and IGF1 were activated by hyaluronic acid products. The in vitro study showed similar effects on tested genes despite a different concentration of hyaluronic acid contained in the medical devices and the simultaneous presence of other additives. Based on the reported data, gene activations are an aspect of metabolic modulation of signalling pathways rather than the proportional production of a specific connective tissue molecule. Indeed, different HABR concentration and the presence of other additives did not change the overall effect on the studied genes. We believe that the optimization of extracellular matrix micro-environment, obtained by enhanced structural support with HABR, leads to functional and metabolic improvement.

Hyaluronic acid in bio-revitalization (HABR), a natural polysaccharide consisting of a linear chain of glucuronic acid and N-acetylglucosamine molecules, is synthesized by the serosa glands of the submucosa and it is an important component of the extracellular matrix of connective tissue, synovial fluid, embryonic mesenchymal tissue, the vitreous humour, the skin and many other organs and tissues in the human body (1-6).

HABR is used in different concentrations in bio-revitalization procedures and in different dermal fillers after crosslinking to reduce its hyaluronidase sensitivity (7-12). HABR is a polymer of dimeric units of N-acetyl-glucosamine and glucuronic acid synthesized by HABR synthetase on the inner surface of plasma membrane, and then secreted in extracellular space.

The purpose of this study is to investigate the role of HABR in bio-revitalization. To this aim, some fibroblastic expressed genes after exposition to three medical devices were evaluated. These products contain different concentrations of HA and are commonly used in aesthetic medicine.

MATERIALS AND METHODS

Primary human dermal fibroblast cell (HFb) culture

Fragments of dermal tissue of healthy volunteers were collected during surgery. The pieces were transferred to 75 cm^2 culture flasks containing DMEM medium (Sigma-Aldrich, Inc., St. Louis, Mo) supplemented with 20% foetal calf serum and antibiotics, i.e., penicillin 100 U/ml and streptomycin 100 µg/ml (Sigma Aldrich, Inc.).

Cells were incubated in a humidified atmosphere of

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5% CO_2 at 37°C. Medium was changed the next day and twice a week. After 15 days, the pieces of dermal tissue were removed from the culture flask. Cells were harvested after additional 24 h of incubation.

Cell cultures

Human dermal fibroblasts at the second passage were seeded on a layer of three different medical devices:

1 - solution of 6.2 mg/ml of non-reticulated HABR of biotechnological origin with a buffered medium containing ammonium molybdate, ammonium metavanadate, calcium chloride, iron sulphate, potassium chloride, copper sulphate, magnesium chloride, manganese sulphate, sodium acetate, sodium hydrogen carbonate, sodium chloride, sodium hydrogen phosphate, sodium metasilicate, sodium selenite, nichel chloride, tin chloride, zinc sulphate, alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glicine, histidine, isoleucine, leucine, lysine, methionine, phenilalanine, proline, serine, threonine, triptophan, tyrosine, valine, adenine (Vit. B4), biotin (Vit. B8), calcium pantothenate (Vit. B5), choline chloride, folic acid (Vit. B9), inositol (Vit. B7), nicotinamide (Vit. B3), pyridoxine (Vit. B6), riboflavin (Vit. B2), thiamine (Vit. B1), vitamin B12, deoxythymidine, glucose, putrescine, sodium pyruvate, lipoic acid, (Viscoderm Skinkò E Ibsa// Revitacare, Saint Ouen d'Aumone, France);

2 - HABR sodium salt 10 mg/ml with a buffered medium containing polynucleotides 10 mg/ml, mannitol, sodium phosphate monobasic dihydrate, sodium phosphate dibasic dehydrate (Newest Mastelli, Sanremo, Italy);

3 - Stabilized HABR gel 20 mg/ml in saline solution phosphate buffered (Restylane Vital, Uppsala, Sweden).

A set of untreated cells was used as control. The cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C for 24 h. After the end of the exposure time, cells were trypsinized and lysed for RNA extraction.

RNA processing and Real Time Polymerase Chain Reaction

Reverse transcription to cDNA was performed directly from cultured cell lysate using the TaqMan Gene Expression Cells-to-Ct Kit (Ambion Inc., Austin, TX), following the 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.).

Finally, the cDNA was amplified by Real Time Polymerase Chain Reaction (PCR). The amplification was performed by using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), and the specific assay was designed for the investigated genes. SYBER assay reactions were performed in a 20 µl volume using the ABI PRISM 7500 (Applied Biosystems). Each reaction contained 10 µl 2´ Power SYBR Green PCR Master Mix (Applied Biosystems), 400 nM concentration of each primer and cDNA.

All experiments performed included non-template controls to exclude contamination of reagents. PCR was performed with two biological replicates.

Expression was quantified using Real Time PCR. The gene expression levels were normalized to the expression of the housekeeping gene Homo sapiens transferrin receptor protein 1 (TFRC).

RESULTS

The effects of the three different medical devices on the primer HAS1 and HYAL1 show an up-regulation of both genes.

DISCUSSION

HABR is an important component of the extracellular matrix of connective tissue, synovial fluid, embryonic mesenchymal tissue, the vitreous humour, the skin and many other organs and tissues in the human body. It is a polysaccharide consisting of a linear chain of glucuronic acid and N-acetylglucosamine molecules, which is synthesized by the serosa glands of the submucosa (13-19).

Low-molecular-weight hyaluronic acid (<300 kD) promotes the stimulation of cell proliferation and has an anti-inflammatory action, while increasing evidence suggests that high-molecular-weight hyaluronic acid (1000 kD), due to its inherent immunosuppressive effect, also helps to regulate exacerbation of symptoms caused by excessive inflammation. HABR is widely used in aesthetic medicine to improve aesthetic surgery results. Firstly, it is non-antigenic, yet it is antioxidant, anti-oedematous and biocompatible. Moreover, it

is bacteriostatic and anti-inflammatory, and thus it prevents the selection and formation of resistant bacterial strains, even when used over an extended period (20-33), which is one of the most significant side effects that occur in the chronic use of antibiotics, making it especially useful in the management of chronic diseases, particularly in respiratory disorders.

HABR plays a key role in wound healing, interacting with type I and II toll-like receptors, it regulates the body's innate immunity against bacteria and viruses by preventing penetration, promoting angiogenesis and by modulating the proliferation, migration and differentiation of the cells responsible for tissue repair (34-43).

In conclusion, the in vitro testing of the three bio-revitalization commercial products determined an up-regulation of genes responsible for HABR synthesis and degradation, for Metalloproteinases 2 and 3 activations, for Desmoplakin production and for metabolic signaling (GDF 6, IGF1) (44-50).

Bio-revitalization procedures have a similar effect despite different HABR concentrations and the concomitant presence of other molecules (i.e., amino acids, vitamins, polynucleotides) which are different in quality and amount (51-56). Only HABRS1 gene shows a different modulation by using the 3 products.

REFERENCES

1. Scarano A, Sbarbati A, Amore R, Iorio EL, Ferraro G, Marchetti M, Amuso D. The role of hyaluronic acid and amino acid against the aging of the human skin: a clinical and histological study. J Cosmet Dermatol 2020; Oct 22. Online ahead of print.
2. Cabral LRB, Teixeira LN, Gimenez RP, Demasi APD, de Brito Junior RB, de Araújo VC, Martinez EF. Effect of Hyaluronic Acid and Poly-L-Lactic Acid Dermal Fillers on Collagen Synthesis: An in vitro and in vivo Study. Clin Cosmet Investig Dermatol 2020; 13:701-710.
3. Sun Z, Zhao H. Comparison of Volume Retention and Biocompatibility of Acellular Dermal Matrix/ Hyaluronic Acid Filler to Autologous Fat Grafts in a Mouse Model. Aesthetic Plast Surg 2020; Oct 13. Online ahead of print.
4. Farollch Prats L, Mirada Donisa E, Villanueva C. "WAY": A practical means to identify and treat the aging process. J Cosmet Dermatol 2020; Oct 12. Online ahead of print.
5. Nguyen TT. Plastic Surgery and Cosmetic Procedures: Facial Injection Procedures. FP Essent 2020; 497:11-17.
6. Lauritano D, Moreo G, Limongelli L, Palmieri A, Carinci F. Drug-Induced Gingival Overgrowth: The Effect of Cyclosporin A and Mycophenolate Mophetil on Human Gingival Fibroblasts. Biomedicines 2020; 8(7):221.
7. Lauritano D, Palmieri A, Lucchese A, Di Stasio D, Moreo G, Carinci F. Role of Cyclosporine in Gingival Hyperplasia: An In Vitro Study on Gingival Fibroblasts. Int J Mol Sci 2020; 21(2):595.
8. Lauritano D, Moreo G, Limongelli L, Tregambi E, Palmieri A, Carinci F. Drug-Induced Gingival Overgrowth: A Pilot Study on the Effect of Diphenylhydantoin and Gabapentin on Human Gingival Fibroblasts. Int J Environ Res Public Health 2020; 17(21):E8229.
9. Di Stasio D, Lauritano D, Iquebal H, Romano A, Gentile E, Lucchese A. Measurement of Oral Epithelial Thickness by Optical Coherence Tomography. Diagnostics (Basel) 2019; 9(3):90.
10. Lico S, Andrisani C, Bassi MA, Candotto V, Silvestre FJ, Lauritano D. Computer-guided implant insertion in a patient with impacted maxillary canines: Case report. J Biol Regul Homeost Agents 2017; 31(2, Suppl.1):247-251.
11. Bassi MA, Andrisani C, Lope, MA, Gaudio RM, Lombardo L, Lauritano D. Guided bone regeneration in distal mandibular atrophy by means of a preformed titanium foil: A case seriesJ Biol Regul Homeost Agents 2016; 30(2):61-68.
12. Lopez MA, Bassi MA, Confalone L, Carinci F, Ormianer Z, Lauritano D. The use of resorbable cortical lamina and micronized collagenated bone in the regeneration of atrophic crestal ridges: A surgical technique. Case series. J Biol Regul Homeost Agents 2016; 30(2):81-85.
13. Carosi P, Barlattani A, Lorenzi C, Bianchi N, Arcuri C. Diode laser as an adjunct to nonsurgical chronic periodontitis therapy: A review. J Biol Regul Homeost Agents 2020; 34(3):45-54.
14. Testi D, Nardone M, Melone P, Ottria L, Arcuri C. HPV and oral lesions: Preventive possibilities,

vaccines and early diagnosis of malignant lesions. Oral Implantol (Rome) 2016; 8(2-3):45-51.
15. Germano F, Germano F, Piro M, Arcuri C, Ottria L. Clinical protocol with digital CAD/CAM chairside workflow for the rehabilitation of severely worn dentition patients. Oral Implantol (Rome) 2017; 10(3):247-261.
16. Lio F, Ottria L, Mazzetti V, Leggeri A, Casella S, Arcuri L. The effectiveness of subgingival irrigant ozone-based as adjuvant for non-surgical periodontal therapy in the treatment of chronic periodontitis: A review. J Biol Regul Homeost Agents 2020; 34(3):27-34.
17. Pirelli P, Fanucci E, Giancotti A, Di Girolamo M, Guilleminault C. Skeletal changes after rapid maxillary expansion in children with obstructive sleep apnea evaluated by low-dose multi-slice computed tomography. Sleep Medicine 2019; 60:75-80.
18. Arcuri C, Petro E, Sollecchia G, Mummolo S, Marzo G. Laser in periodontal pockets: In vivo and in vitro study. J Biol Regul Homeost Agents 2020; 34(3):139–146.
19. Campanella V, Di Taranto V, Beretta M, Colombo S, Gallusi G. Paediatric endodontics. Part. 1: Portland Cements Apical Plug Eur J Paediatr Dent 2020; 21(3):248-250.
20. Milia E, Usai M, Szotáková B, Elstnerová M, Králová V, D'hallewin G, Spissu Y, Barberis A, Marchetti M, Bortone A, Campanella V, Mastandrea G, Langhansová L, Eick S. The Pharmaceutical Ability of Pistacia lentiscus L. Leaves Essential Oil Against Periodontal Bacteria and Candida sp. and Its Anti-Inflammatory Potential. Antibiotics (Basel) 2020; 9(6):281.
21. Usai P, Campanella V, Sotgiu G, Spano G, Pinna R, Eramo S, Saderi L, Garcia-Godoy F, Derchi G, Mastandrea G, Milia E. Effectiveness of Calcium Phosphate Desensitising Agents in Dental Hypersensitivity Over 24 Weeks of Clinical Evaluation. Nanomaterials (Basel) 2019; 9(12):1748.
22. Quinzi V, Mummolo S, Bertolazzi F, Campanella V, Marzo G, Marchetti E. Comparison of Mandibular Arch Expansion by the Schwartz Appliance Using Two Activation Protocols: A Preliminary Retrospective Clinical Study. J Funct Morphol Kinesiol 2020; 5(3):61.
23. Beretta M, Canova FF, Moscati M, Campanella V, Gallusi G. State-of-the-art on MIH. Part 2 MIH clinical management using ozone. Eur J Paediatr Dent 2020; 21(2):163-166.
24. Libonati A, Montella D, Montemurro E, Campanella V. External cervical resorption: a case report. Eur J Paediatr Dent 2017; 18(4):296-298.
25. Esin S, Pasini M, Miceli M, Cosseddu G, Giuca MR, Batoni G. Longitudinal study on the effect of oral hygiene measures on the salivary count of microbial species with cariogenic potential. J Biol Regul Homeost Agents 2018; 32(6):1407-1420.
26. Miceli M, Cosseddu G, Pasini M, Semeraro S, Lardani L, Giuca MR. Simplified basic periodontal examination in adolescents before and after a tailored treatment dental program. Minerva Stomatol 2020; 69(2):72-78.
27. Giuca MR, Miceli M, Carli E, Lardani L, Marchio V, Baldini C. Impact of Sjögren's syndrome on oral health and quality of life: an observational cross-sectional study. J Biol Regul Homeost Agents 2020; 34(3 Suppl.1):129-137
28. Marchetti E, Petro E, Gaggioli F, Lardani L, Mancini L, Marzo G. The dentist's role in diagnosis and treatment of obstructive sleep apnea syndrome: a literature review. J Biol Regul Homeost Agents 2020; 34(3 Suppl. 1):173-180.
29. Pasini M, Giuca MR, Ligori S, Mummolo S, Fiasca F, Marzo G, Quinzi V. Association between Anatomical Variations and Maxillary Canine Impaction: A Retrospective Study in Orthodontics. Appl Sci 2020; 10(16):5638.
30. Mazza D, Di Girolamo M, Cecchetti F, Baggi L. MRI findings of working and non-working tmj during nilateral molar clenching on hard bolus. J Biol Regul Homeost Agents 2020; 34(3 Suppl. 1):1-8.
31. Libonati A, Montemurro E, Nardi R, Campanella V. Percentage of Gutta-percha-filled Areas in Canals Obturated by 3 Different Techniques with and without the Use of Endodontic Sealer. J Endod 2018; 44(3):506-509.
32. Campanella, V., Mummolo, S., Grazzini, F., Barlattani, A., Di Girolamo, M. The effectiveness of endodontic sealers and endodontic medicaments on the elimination of Enterococcus faecalis: An in vitro study Journal of Biological Regulators and

Homeostatic Agents, 2019; 33(3):97–102.
33. Marchetti E, Mummolo S, Mancini L, Quinzi V, Pontieri E, Marzo G, Campanella V. Decontamination in the dental office: a comparative assessment of a new active principle. Dental Cadmos, 2021; 89(3):200–206.
34. Mazza D, Di Girolamo M, Cecchetti F, Baggi L. Appearance of normal MRI anatomy of the lingual nerve using steady-state free precession sequences at 3-T. J Biol Regul Homeost Agents 2020; 34(3 Suppl. 1):19-26.
35. Cecchetti F, Di Girolamo M, Ippolito DG, Baggi L. Computer-guided implant surgery: analysis of dynamic navigation systems and digital accuracy. J Biol Regul Homeost Agents 2020; 34(3 Suppl. 1):9-17.
36. Ruggiero F, Carbone D, Mugavero R, Palmieri A, Lauritano D, Baggi L, Nardone M, Martinelli M, Carinci F. Human polyomavirus in tonsillar microbiota of an Afghan population group. J Biol Regul Homeost Agents 2018; 32(2 Suppl. 1):185-190.
37. Ottria L, Candotto V, Cura F, Baggi L, Arcuri C, Nardone M, Gaudio RM, Gatto R, Spadari F, Carinci F. HPV acting on E-cadherin, p53 and p16: literature review. J Biol Regul Homeost Agents 2018; 32(2 Suppl. 1):73-79.
38. Carinci F, Scapoli L, Contaldo M, Santoro R, Palmieri A, Pezzetti F, Lauritano D, Candotto V, Mucchi D, Baggi L, Tagliabue A, Tettamanti L. Colonization of Legionella spp. in dental unit waterlines. J Biol Regul Homeost Agents 2018; 32(2 Suppl. 1):139-142.
39. Mancini L, Quinzi V, Mummolo S, Marzo G, Marchetti E. Angiotensin-converting enzyme 2 as a possible correlation between COVID-19 and periodontal disease. Appl Sci (Switzerland) 2020; 10(18):6224.
40. Saccomanno S, Quinzi V, Sarhan S, Laganà D, Marzo G. Perspectives of tele-orthodontics in the COVID-19 emergency and as a future tool in daily practice. Eur J Paediatr Dent 2020; 21(2):157–162.
41. Quinzi V, Saccomanno S, Manenti RJ, Giancaspro S, Coceani L, Marzo G. Efficacy of rapid maxillary expansion with or without previous adenotonsillectomy for pediatric obstructive sleep apnea syndrome based on polysomnographic data: A systematic review and meta-analysis. Appl Sci (Switzerland) 2020; 10(18):2820.
42. Mummolo S, Mancini L, Quinzi V, D'Aquino R, Marzo G, Marchetti E. Rigenera® autologous micrografts in oral regeneration: Clinical, histological, and radiographical evaluations. Appl Sci (Switzerland) 2020; 10(15):5084.
43. Quinzi V, Tecco S, Nota A, Mummolo S, Marzo G. Mesial rotation of the upper first molar: Association with anterior dental crowding in mixed and permanent dentition. Appl Sci (Switzerland) 2020; 10(15):5301.
44. Quinzi V, Salvatorelli C, Panetta G, Rizzo FA, Mummolo S. Autotransplatation of immature third molars as substitutes for congenitally missing second premolars: An alternative solution in a young patient with oligodontia. J Biol Regul Homeost Agents 2020; 34(3):155–163.
45. Daniele V, Macera L, Taglieri G, Di Giambattista A, Spagnoli G, Massaria A, Messori M, Quagliarini E, Chiappini G, Campanella V, Mummolo S, Marchetti E, Marzo G, Quinzi V. Thermoplastic Disks Used for Commercial Orthodontic Aligners: Complete Physicochemical and Mechanical Characterization. Materials (Basel). 2020 May 22;13(10):2386.
46. Marchetti E, Mancini L, Bernardi S, ...Marzo G, Macchiarelli G. Evaluation of different autologous platelet concentrate biomaterials: Morphological and biological comparisons and considerations Materials 2020; 13(10):2282.
47. Mummolo S, Nota A, Albani F, ...Quinzi V, Tecco S. Salivary levels of Streptococcus mutans and Lactobacilli and other salivary indices in patients wearing clear aligners versus fixed orthodontic appliances: An observational study. PLoS ONE 2020; 15(4):e0228798.
48. Giuca MR, Pasini M, Pacini M, Carli E, Lardani L, Ferro R. Use of extra-oral scanner for the study of arch form in a sample of Italian adolescents with ideal occlusion. J Biol Regul Homeost Agents 2020; 34(3 Suppl. 1):107-116.
49. Nastasio S, Sciveres M, Maggiore G. The Best Choice for Second-line Agent in Standard Treatment-refractory Children with Autoimmune Hepatitis. J Pediatr Gastroenterol Nutr 2018; 66(3):e86-e87.
50. Marchetti E, Casalena F, Capestro A, ...Mattei A, Marzo G. Efficacy of two mouthwashes on 3-day supragingival plaque regrowth: a randomized crossover clinical trial. Int J Dent Hyg 2017;

15(1):73–80.
51. Rosa M, Quinzi V, Marzo G. Paediatric Orthodontics Part 1: Anterior open bite in the mixed dentition. Eur J Paediatr Dent 2019; 20(1):80–82.
52. Paolantonio EG, Ludovici N, Saccomanno S, La Torre G, Grippaudo C. Association between oral habits, mouth breathing and malocclusion in Italian preschoolers. Eur J Paediatr Dent 2019; 20(3):204–208.
53. Saccomanno S, Di Tullio A, D'Alatri L, Grippaudo C. Proposal for a myofunctional therapy protocol in case of altered lingual frenulum. A pilot study. Eur J Paediatr Dent 2019; 20(1):67–72.
54. Quinzi V, Scibetta ET, Marchetti E, Mummolo S, Giannì AB, Romano M, Beltramini G, Marzo G. Analyze my face. Journal of Biological Regulators and Homeostatic Agents 2018, 32(2):149–158.
55. Silvestrini-Biavati A, Angiero F, Gambino A, Ugolini A. Do changes in spheno-occipital synchondrosis after rapid maxillary expansion affect the maxillomandibular complex? Eur J Paediatr Dent 2013;14(1):63-7.
56. Mongini F, Evangelista A, Rota E, Ferrero L, Ugolini A, Ceccarelli M, Ciccone G, Galassi C. Long-term benefits of an educational and physical program on headache, and neck and shoulder pain, in a working community. J Pain 2009;10(11):1138-45.