Living Cell-based Regenerative Medicine Technologies for Periodontal Soft Tissue Augmentation

Michael K. McGuire *†‡§, DDS, Lorenzo Tavelli *†, DDS,
Stephen E. Feinberg ‖, DDS, MS, PhD, Giulio Rasperini †¶, DDS,
Giovanni Zucchelli †#, DDS, PhD, Hom-Lay Wang †, DDS, MS, PhD,
William V. Giannobile †**, DDS, MS, DMSc

† Department of Periodontics & Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI, USA

‡ Private practice, Houston, TX, USA

§ Department of Periodontics, University of Texas, Dental Branch Houston and Health Science Center at San Antonio, TX, USA

‖ Department of Oral and Maxillofacial Surgery, University of Michigan, Ann Arbor, MI, USA

¶ Department of Biomedical, Surgical and Dental Sciences, University of Milan, Foundation IRCCS Ca' Granda Policlinic, Milan, Italy

Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

** Department of Biomedical Engineering and Biointerfaces Institute, College of Engineering, University of Michigan, Ann Arbor, MI, USA

* Contributed equally to this article

Correspondence

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/JPER.19-0353.

William V. Giannobile, DDS, MS, DMSc
Najjar Professor of Dentistry and Chair, Department of Periodontics and Oral Medicine
University of Michigan, School of Dentistry
1011 North University Avenue
Ann Arbor, Michigan 48109-1078, USA.
E-mail address: wgiannob@umich.edu

2518 Words; 4 Figures; 1 Table; 46 References

Running Title: Living cells-based technologies for periodontal plastic surgery

One Sentence Summary: Living cellular constructs demonstrate clinical safety and efficacy for use in augmenting keratinized tissue width and root coverage.

Abstract

The cultivation of human living cells into scaffolding matrices has progressively gained popularity in the field of periodontal wound healing and regeneration. Living constructs based on fibroblasts, keratinocytes alone or in combination have been developed and used as alternatives to autogenous soft tissue grafts in keratinized tissue (KT) augmentation and in root coverage procedures. Their promising advantages include reduced patient morbidity, unlimited graft availability, and comparable esthetics. This manuscript reviews soft tissue augmentation and root coverage procedures using bioengineered living cellular therapy and highlights their expected clinical, esthetic and patient-related outcomes.

Keywords: tissue engineering, tissue scaffolds, periodontal, soft tissue grafting, gingival recession, regenerative medicine

Tissue engineered constructs (TECs)

The implantation of living cells in scaffold materials (tissue engineered constructs, TECs) has represented a new line in the field of soft tissue grafting. It has been suggested that one of the main advantages of living cell-based technology is the ability to communicate with the host by modulating cytokine expression [1, 2]. Bioengineered living cellular therapy can be classified based on the cell types contained in the carrier matrices. This review aims to present the characteristics and clinical application of cell-based constructs for root coverage and soft tissue augmentation.

Fibroblast-based constructs

Living human dermal replacement graft †† is manufactured through the three-dimensional cultivation of neonatal human fibroblasts on a bioabsorbable polyglactin mesh [3, 4]. The scaffold matrix degrades by hydrolysis and is lost after transplantation, leaving the extracellular matrix component and fibroblasts which secrete growth factors (GFs) and other proteins, including human dermal collagen, fibronectin, glycosaminoglycans and cytokines [3-5]. This process results in a living metabolically active dermal structure that promotes the colonization of the wound by adjacent cells, angiogenesis and re-epithelialization [3, 5]. The dermal replacement graft acts both as a scaffold, encouraging the attachment and migration of keratinocytes, and as a wound healing agent[4].

This construct has been extensively used in the treatment of neuropathic diabetic foot ulcers [3, 5, 6] where it was found to be effective in promoting a faster healing and a higher chance of complete wound closure than conventional treatments (i.e., skin grafting, wound dressings, or local growth factor application), with no differences in the incidence of adverse effects [7-9]. Because of its properties, the dermal replacement graft was introduced in

periodontal plastic surgery for soft tissue augmentation [4]. More recent studies have addressed the outcomes of autologous gingival fibroblasts seeded in acellular scaffolds, such as collagen matrix (CM) [10], acellular dermal matrix (ADM) [11] or hyaluronic acid scaffold [12], in treating GRs or increasing keratinized tissue (KT) width.

Keratinocyte-based constructs

Ex vivo-produced oral mucosal equivalent (EVPOME) is a living cellular construct composed by autogenous keratinocytes, obtained from a punch biopsy then purified and cultivated on ADM ‡‡ [13, 14]. The ADM and the keratinocytes are immersed within a cell culture media with the necessary signaling molecules to push their development along the desired path [15]. The entire process for obtaining an EVPOME from a harvesting site from the patient takes less than one month and requires strict current Good Manufacturing Practices (cGMP) [16]. EVPOME expresses differentiation (filaggrin and cytokeratin 10/13) and proliferation (proliferating cell nuclear antigen and Ki-67) markers, suggesting an early-stage and active keratinization and proliferative process [13]. EVPOME exhibits a monolayer composed by seeded keratinocytes over the ADM in the first 4 days, while a continuous stratified and well-differentiated epidermis on the dermal matrix was observed after 11-18 days [14]. Recently, it has been reported that ADM biological and physical characteristics affect the epithelial maturation of the EVPOME [17, 18]. Furthermore, this TEC can modulate the inflammatory response by releasing GFs (including keratinocytes and vascular endothelial growth factors [VEGF]) and promoting early vascular invasion and revascularization [19, 20]. Therefore, EVPOME has been used in the treatment of intraoral mucosal grafting for KT width augmentation [16] and for mucosal reconstruction after the excision of oral lesions or in situations with deficient keratinized attached gingiva [20, 21] (Figures 1 and 2).

Khmaladze and coworkers recently proposed a non-invasive method that allows real-time monitoring of the thermal stress, and therefore the viability, of the EVPOME prior to implantation [22]. The same group demonstrated that high levels of interleukin-8 (IL-8), human β-defensin I (hBD-I) and tissue inhibitor of metalloproteinase 2 (TIMP-2) were predictors of healthy EVPOME [23]. Nevertheless, further clinical studies are needed, as this method appears promising not only for distinguishing stress and non-stressed EVPOME before implantation but also for evaluating post-grafted outcomes [22, 24].

Fibroblast and Keratinocyte-based combination constructs

Living Cellular Construct (LCC) §§ consists of a 3D bovine collagen matrix seeded with keratinocytes and dermal fibroblasts derived from human neonatal foreskin [25, 26]. LCC was the first allogenic cell-based graft approved by Food and Drug Administration (FDA) and it has been shown to enhance wound healing and likelihood of complete wound closure in chronic wounds, diabetic foot ulcers, and venous leg ulcers [25, 26]. The rationale behind using a construct based on two cell types is that dermal fibroblasts are responsible for the homeostasis of the extracellular matrix, which is crucial for keratinocytes growth and differentiation, while keratinocytes form the external epithelial layer and provide a barrier effect. One of the main advantages of LCC is the paracrine signaling, known as cross-talk, between keratinocytes and fibroblasts that play a key role during the healing of the LCC [26]. Indeed, it has been observed that the expression of cytokines and growth factors modulated by LCC, including bone morphogenetic protein (BMP)s, fibroblast growth factor (FGF)-11, Insulin like growth factor (IGF)-1, platelet derived growth factor (PDGF) and VEGF, differs

from other TECs-based on one cell type only [26], suggesting that both keratinocytes and fibroblasts are required to reproduce a fully-developed epithelium [26].

Clinical outcomes of cell-based tissue engineered constructs in periodontal plastic surgery

Preclinical studies were designed to evaluate not only the efficacy and safety of TECs, but also for assessing their interactions with the host tissues via histological and histomorphometric analyses [27-29]. It was demonstrated that the incorporation of keratinocytes and/or fibroblasts on acellular scaffolds is well tolerated by the host and can enhance blood vessel formation and cells migration by secreting specific growth GFs [10, 18, 27-30]. Similarly, the efficacy in the early phases of healing of autologous cultured and expanded fibroblasts in the treatment of interdental papillary defect has been also described [31].

Keratinized tissue width augmentation

Pini-Prato and coworkers were pioneers that investigated the use of TECs in periodontal plastic surgery [12, 32]. In six patients requiring KT augmentation, autologous human fibroblasts were obtained from the gingivae and cultured on a non-woven matrix of benzyl ester of hyaluronic acid (HA). The graft was adapted and stabilized over the exposed periosteum with sutures. The authors observed a granulation-like tissue during the first 2 weeks, while the graft was no longer detectable after 1 month. After 3 months, the grated site appeared epithelialized with an average KT width gain of 2 ± 0.4 mm [12].

McGuire et al. performed a series of studies aiming at evaluating whether TECs can be considered a safe and a viable alternative to autogenous FGG in KT width augmentation [1, 2, 4]. They designed the first randomized clinical trial (RCT) comparing a TECs containing

human allogenic fibroblasts †† to free gingival graft (FGG)[4]. According to the authors, the use of fibroblasts without keratinocytes did not affect the keratinization of the gingival epithelium, speculating that GFs secreted by the TEC can positively influence the growth of the keratinocyte layer. In line with this observation, biopsies from both groups collected at 6 months showed similar connective tissue covered by keratinized epithelium and that the connective tissue layer of the TEC appeared more organized than FGG one. The dermal replacement graft showed a significant shrinkage that contributed to an inferior KT width gain (2.7 mm on average) than FGG, which exhibited an average of 1-1.2 mm greater KT width[4] (Figures 3A through 3D).

Later on, the same authors investigated the safety and effectiveness of an LCC containing fibroblasts and keratinocytes §§. While the pilot study provided promising results supporting the ability of LCC to regenerate KT and attached gingiva without the morbidity of an additional surgical site [2], the multi-center RCT including 96 patients further confirmed and extended the findings from the previous study [1]. After 6-months, LCC was able to regenerate at least 2 mm of KT width in 95% of patients, although the overall KT width gain was inferior than that observed following FGG (3.2 ± 1.1 mm vs 4.6 ± 1 mm, respectively). This result seems particularly crucial since an ideal alternative graft material should be able to regenerate at least 2 mm of KT while providing comparable or superior patient-reported outcomes [1,33] (Figure 4).

The authors reported also that, while site grafted with an FGG tended to retain the characteristics of the palatal tissue, sites that received LCC showed statistically significant superior esthetic results, in terms of color match and texture, when compared to adjacent tissues [1]. The authors then speculated that the greater esthetic results of LCC was probably

due to the fact that the material acts not as a graft but more as a cell-delivery therapy encouraging the adjacent native cells to migrate into and over it [1, 2]. This stimulation of native cells mediated by the secretion of GFs and cytokines may be responsible for the generation of a site-appropriate tissue [1, 2, 34]. In addition, it was observed an up-regulation of angiogenic-related biomarkers, such as angiogenin, angiostatin, PDGF-BB, VEGF, FGF-2, Interleukin (IL)-8, Tissue inhibit of metalloproteinase (TIMP)-1, TIMP-2, Granulocyte-macrophage colony-stimulating factor (GM-CSF) and Interferon Gamma-Induced Protein 10 (IP-10), in LCC group compared to FGG at the early stage of wound healing [35]. Furthermore, most patients preferred the LCC treatment than FGG[1] with no adverse events reported. The authors concluded that LCC may be considered a safe and an effective alternative to the FGG for augmenting attached gingivae, especially when a major is to avoid palatal autogenous tissue and to regenerate a site-appropriate tissue [1, 2]. Similar results in terms of safety and regeneration of a site-appropriate tissue were also demonstrated by Nevins [36]. The DNA persistence analysis did not reveal the presence of the LCC in the site after 3 to 7 weeks, supporting the hypothesis that the construct acts as a local wound healing agent and not as a graft, guiding the patients' own cells to develop new tissue which matches the surrounding gingiva [36]. Another group evaluated the efficacy of a TEC containing human autologous keratinocytes harvested from the palate, which were expanded and then cultured on a ADM (EVPOME) [16]. EVPOME was positioned on a partial-thickness flap and secured to the surrounding gingiva and underlying periosteum with sutures. After 6 months, the treated sites exhibited a mean KT gain of 3 mm, without any significant adverse events during follow-up [16].

Root coverage procedures

Xenogeneic and human-derived scaffolds failed to provide the same outcomes of autogenous connective tissue graft (CTG) in terms of root coverage [37, 38]. Therefore, researchers have started to investigate the adjunct of living cells (fibroblasts or stem cells) in combination with acellular scaffolds. TECs can be based on patient's autologous cells [10, 11, 39, 40], allogenic cells from newborn foreskin or umbilical cord [41-43]. Wilson et al. were among the first to investigate the use of dermal replacement graft †† as a substitute of CTG in root coverage procedure [41] (Figures 3E through 3F). While dermal replacement graft showed inferior results as compared to FGG when used for KT augmentation [4], the study showed similar results between the TEC and CTG, in terms of mean root coverage, KT width gain, patient satisfaction and esthetics [41]. The authors also highlighted that clinical handling characteristics of dermal replacement graft was more favorable than CTG. It was observed that complete root coverage with the TEC was obtained only when the material was completely covered by the flap and not when it was left partially exposed, suggesting despite the fact that dermal replacement graft is a metabolically active graft with angiogenic activity, it cannot survive over avascular root surface without the double blood supply of the flap [41].

Later, several clinicians described the use of LCCs with autogenous fibroblasts harvested weeks prior to the surgery [10, 11, 39, 40]. In a case series study, it was obtained a mean root coverage (mRC) of 79.1% and a KT width gain of 1.1 mm using CAF + cultured gingival dermal substitute composed of autologous fibroblast harvested from the retromolar region of the mandible and seeded in a two layered matrix of hyaluronic acid sponge and atelo-collagen gel [39]. When the TEC was compared to the acellular scaffold itself [10, 11], a study did not find any significant differences in terms of mRC and KT width gain between ADM and ADM seeded with autologous fibroblasts [11], while another group reported a mRC of 69.6% and 38.3% for autologous fibroblast seeded on a collagen matrix and collagen

matrix alone, respectively [10]. The reason for these contrasting results is open to speculations. It may be reasonable to assume that case selection (type of GRs), region of harvesting, cells culture and scaffold, and patient behavior may have contributed to these conflicting outcomes.

Milinkovic et al. obtained a similar mRC (89.9% vs 91.3%) and root coverage esthetic score (8.67 vs 8.61) between CTG and TEC based on cultured autogenous fibroblast on a collagen matrix, respectively. However, CTG achieved more KT width gain than the TEC (2.26 mm vs 1.74 mm) [40].

It has been reported that bone marrow-derived mesenchymal stem cells (MSCs) have the property of enhancing periodontal regeneration by differentiating into fibroblasts, cementoblasts and osteoblasts [44, 45]. In particular, MSCs can be isolated from umbilical cord tissues, stored frozen and then thawed to provide stem cells. MSCs derived from umbilical cord possesses a high frequency of colony-forming unit-fibroblast (CFU-F)-deriving cells that contribute to promote bone formation [46]. The clinical application of MSCs for the treatment of GRs was investigated in a RCT in which MSCs were cultivated them on a polylactide/polyglycolide (PLA/PGA) scaffold [42]. Compared to CTG, that served as a control, the TEC achieved slightly lower mRC, however a greater CAL gain was observed in sites that received the MSCs + PLA/PGA. The authors speculated that MSCs may have induced a healing with periodontal regeneration rather than repair in the GR defects [42]. In a more recent trial, the same group compared CAF + PLA/PGA scaffold (controls) versus CAF + MSCs cultured on a PLA/PGA scaffold (test), showing statistically superior mRC in controls and, thus, suggesting a positive role of MSCs on root coverage outcomes [43]. Table 1 summarizes the clinical studies that investigated the use of TEC.

At this moment, TECs have not yet been applied to implant dehiscence defect soft tissue coverage.

Concluding remarks

Evidence supports the safety and efficacy of living cellular constructs for use in augmenting keratinized tissues. Improved esthetics, lower morbidity and higher patient preference are among their main advantages as compared to autogenous grafts. Although living cellular constructs may be considered the biomaterial of choice when treating generalized mucosal defects or when the primary aim is to reduce patient morbidity, autogenous soft tissue grafts provide superior clinical outcomes in keratinized tissue width augmentation and root coverage.

Acknowledgements and Conflict of Interest Statement

WVG and MKM have previously consulted for Organogenesis. MKM has received direct research support from Advance Tissue Science, Organogenesis and Isolagen. SEF is a founding member of the Tissue Regeneration Systems (TRS) and chair of the Scientific Advisory Board in Craniomaxillofacial Surgery for TRS. The other authors do not have any financial interests, either directly or indirectly, in the products or information associated with this manuscript. This paper was partially supported by the University of Michigan Periodontal Graduate Student Research Fund.

Footnotes

†† Dermagraft, Advanced Tissue Sciences, Inc., La Jolla, CA, USA

‡‡ AlloDerm, LifeCell, Branchburg, NJ, USA

§§ Gintuit, Organogenesis, Inc., Canton, MA, USA

References

1. McGuire MK, Scheyer ET, Nevins ML, et al. Living cellular construct for increasing the width of keratinized gingiva: results from a randomized, within-patient, controlled trial. *J Periodontol* 2011;82:1414-1423.

2. McGuire MK, Scheyer ET, Nunn ME, Lavin PT. A pilot study to evaluate a tissue-engineered bilayered cell therapy as an alternative to tissue from the palate. *J Periodontol* 2008;79:1847-1856.

3. Gentzkow GD, Iwasaki SD, Hershon KS, et al. Use of dermagraft, a cultured human dermis, to treat diabetic foot ulcers. *Diabetes Care* 1996;19:350-354.

4. McGuire MK, Nunn ME. Evaluation of the safety and efficacy of periodontal applications of a living tissue-engineered human fibroblast-derived dermal substitute. I. Comparison to the gingival autograft: a randomized controlled pilot study. *J Periodontol* 2005;76:867-880.

5. Mansbridge J, Liu K, Patch R, Symons K, Pinney E. Three-dimensional fibroblast culture implant for the treatment of diabetic foot ulcers: metabolic activity and therapeutic range. *Tissue Eng* 1998;4:403-414.

6. Naughton G, Mansbridge J, Gentzkow G. A metabolically active human dermal replacement for the treatment of diabetic foot ulcers. *Artif Organs* 1997;21:1203-1210.

7. Marston WA, Hanft J, Norwood P, Pollak R, Dermagraft Diabetic Foot Ulcer Study G. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. *Diabetes Care* 2003;26:1701-1705.

8. Harding K, Sumner M, Cardinal M. A prospective, multicentre, randomised controlled study of human fibroblast-derived dermal substitute (Dermagraft) in patients with venous leg ulcers. *Int Wound J* 2013;10:132-137.

9. Hart CE, Loewen-Rodriguez A, Lessem J. Dermagraft: Use in the Treatment of Chronic Wounds. *Adv Wound Care (New Rochelle)* 2012;1:138-141.

10. Koseoglu S, Duran I, Saglam M, Bozkurt SB, Kirtiloglu OS, Hakki SS. Efficacy of collagen membrane seeded with autologous gingival fibroblasts in gingival recession treatment: a randomized, controlled pilot study. *J Periodontol* 2013;84:1416-1424.

11. Jhaveri HM, Chavan MS, Tomar GB, Deshmukh VL, Wani MR, Miller PD, Jr. Acellular dermal matrix seeded with autologous gingival fibroblasts for the treatment of gingival recession: a proof-of-concept study. *J Periodontol* 2010;81:616-625.

12. Pini Prato GP, Rotundo R, Magnani C, Soranzo C, Muzzi L, Cairo F. An autologous cell hyaluronic acid graft technique for gingival augmentation: a case series. *J Periodontol* 2003;74:262-267.

13. Izumi K, Terashi H, Marcelo CL, Feinberg SE. Development and characterization of a tissue-engineered human oral mucosa equivalent produced in a serum-free culture system. *J Dent Res* 2000;79:798-805.

14. Izumi K, Takacs G, Terashi H, Feinberg SE. Ex vivo development of a composite human oral mucosal equivalent. *J Oral Maxillofac Surg* 1999;57:571-577; discussion 577-578.

15. Kim RY, Fasi AC, Feinberg SE. Soft tissue engineering in craniomaxillofacial surgery. *Ann Maxillofac Surg* 2014;4:4-8.

16. Izumi K, Neiva RF, Feinberg SE. Intraoral grafting of tissue-engineered human oral mucosa. *Int J Oral Maxillofac Implants* 2013;28:e295-303.

17. Kato H, Marcelo CL, Washington JB, Bingham EL, Feinberg SE. Fabrication of Large Size Ex Vivo-Produced Oral Mucosal Equivalents for Clinical Application. *Tissue Eng Part C Methods* 2015;21:872-880.

18. Kuo S, Kim HM, Wang Z, et al. Comparison of two decellularized dermal equivalents. *J Tissue Eng Regen Med* 2018;12:983-990.

19. Xu Q, Izumi K, Tobita T, Nakanishi Y, Feinberg SE. Constitutive release of cytokines by human oral keratinocytes in an organotypic culture. *J Oral Maxillofac Surg* 2009;67:1256-1264.

20. Hotta T, Yokoo S, Terashi H, Komori T. Clinical and histopathological analysis of healing process of intraoral reconstruction with ex vivo produced oral mucosa equivalent. *Kobe J Med Sci* 2007;53:1-14.

21. Izumi K, Feinberg SE, Iida A, Yoshizawa M. Intraoral grafting of an ex vivo produced oral mucosa equivalent: a preliminary report. *Int J Oral Maxillofac Surg* 2003;32:188-197.

22. Khmaladze A, Kuo S, Kim RY, et al. Human oral mucosa tissue-engineered constructs monitored by Raman fiber-optic probe. *Tissue Eng Part C Methods* 2015;21:46-51.

23. Kuo S, Zhou Y, Kim HM, et al. Biochemical indicators of implantation success of tissue-engineered oral mucosa. *J Dent Res* 2015;94:78-84.

24. Chen LC, Lloyd WR, Kuo S, et al. The potential of label-free nonlinear optical molecular microscopy to non-invasively characterize the viability of engineered human tissue constructs. *Biomaterials* 2014;35:6667-6676.

25. Kirsner RS, Sabolinski ML, Parsons NB, Skornicki M, Marston WA. Comparative effectiveness of a bioengineered living cellular construct vs. a dehydrated human amniotic membrane allograft for the treatment of diabetic foot ulcers in a real world setting. *Wound Repair Regen* 2015;23:737-744.

26. Wojtowicz AM, Oliveira S, Carlson MW, Zawadzka A, Rousseau CF, Baksh D. The importance of both fibroblasts and keratinocytes in a bilayered living cellular construct used in wound healing. *Wound Repair Regen* 2014;22:246-255.

27. Novaes AB, Jr., Marchesan JT, Macedo GO, Palioto DB. Effect of in vitro gingival fibroblast seeding on the in vivo incorporation of acellular dermal matrix allografts in dogs. *J Periodontol* 2007;78:296-303.

28. Yoshizawa M, Koyama T, Kojima T, Kato H, Ono Y, Saito C. Keratinocytes of tissue-engineered human oral mucosa promote re-epithelialization after intraoral grafting in athymic mice. *J Oral Maxillofac Surg* 2012;70:1199-1214.

29. Bornstein MM, Reichart PA, Buser D, Bosshardt DD. Tissue response and wound healing after placement of two types of bioengineered grafts containing vital cells in submucosal maxillary pouches: an experimental pilot study in rabbits. *Int J Oral Maxillofac Implants* 2011;26:768-775.

30. Lotfi G, Shokrgozar MA, Mofid R, et al. A clinical and histologic evaluation of gingival fibroblasts seeding on a chitosan-based scaffold and its effect on the width of keratinized gingiva in dogs. *J Periodontol* 2011;82:1367-1375.

31. McGuire MK, Scheyer ET. A randomized, double-blind, placebo-controlled study to determine the safety and efficacy of cultured and expanded autologous fibroblast injections for the treatment of interdental papillary insufficiency associated with the papilla priming procedure. *J Periodontol* 2007;78:4-17.

32. Pini Prato GP, Rotundo R, Magnani C, Soranzo C. Tissue engineering technology for gingival augmentation procedures: a case report. *Int J Periodontics Restorative Dent* 2000;20:552-559.

33. McGuire MK, Scheyer ET, Gwaltney C. Commentary: incorporating patient-reported outcomes in periodontal clinical trials. *J Periodontol* 2014;85:1313-1319.

34. Scheyer ET, Nevins ML, Neiva R, et al. Generation of site-appropriate tissue by a living cellular sheet in the treatment of mucogingival defects. *J Periodontol* 2014;85:e57-64.

35. Morelli T, Neiva R, Nevins ML, et al. Angiogenic biomarkers and healing of living cellular constructs. *J Dent Res* 2011;90:456-462.

36. Nevins ML. Tissue-engineered bilayered cell therapy for the treatment of oral mucosal defects: a case series. *Int J Periodontics Restorative Dent* 2010;30:31-39.

37. Cairo F, Nieri M, Pagliaro U. Efficacy of periodontal plastic surgery procedures in the treatment of localized facial gingival recessions. A systematic review. *J Clin Periodontol* 2014;41 Suppl 15:S44-62.

38. Chambrone L, Tatakis DN. Periodontal soft tissue root coverage procedures: a systematic review from the AAP Regeneration Workshop. *J Periodontol* 2015;86:S8-51.

39. Murata M, Okuda K, Momose M, Kubo K, Kuroyanagi Y, Wolff LF. Root coverage with cultured gingival dermal substitute composed of gingival fibroblasts and matrix: a case series. *Int J Periodontics Restorative Dent* 2008;28:461-467.

40. Milinkovic I, Aleksic Z, Jankovic S, et al. Clinical application of autologous fibroblast cell culture in gingival recession treatment. *J Periodontal Res* 2015;50:363-370.

41. Wilson TG, Jr., McGuire MK, Nunn ME. Evaluation of the safety and efficacy of periodontal applications of a living tissue-engineered human fibroblast-derived dermal substitute. II. Comparison to the subepithelial connective tissue graft: a randomized controlled feasibility study. *J Periodontol* 2005;76:881-889.

42. Zanwar K, Laxmanrao Bhongade M, Kumar Ganji K, S BK, Gowda P. Comparative evaluation of efficacy of stem cells in combination with PLA/PGA membrane versus sub-epithelial connective tissue for the treatment of multiple gingival recession defects: a clinical study. *J Stem Cells* 2014;9:253-267.

43. Zanwar K, Kumar Ganji K, Bhongade ML. Efficacy of Human Umbilical Stem Cells Cultured on Polylactic/ Polyglycolic Acid Membrane in the Treatment of Multiple Gingival Recession Defects: a Randomized Controlled Clinical Study. *J Dent (Shiraz)* 2017;18:95-103.

44. Hasegawa N, Kawaguchi H, Hirachi A, et al. Behavior of transplanted bone marrow-derived mesenchymal stem cells in periodontal defects. *J Periodontol* 2006;77:1003-1007.

45. Kawaguchi H, Hirachi A, Hasegawa N, et al. Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. *J Periodontol* 2004;75:1281-1287.

46. Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE. Human umbilical cord perivascular (HUCPV) cells: a source of mesenchymal progenitors. *Stem Cells* 2005;23:220-229.

Author Manuscript

Tables

Table 1. Characteristics and outcomes of clinical studies evaluating the safety and efficacy of tissue engineered constructs in keratinized tissue width augmentation and root coverage.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/JPER.19-0353.

Publication	Study design	Aim	Cells type and origin	Cells culture medium	Scaffold	Test group (number of sites)	Control group (number of sites)	Clinical outcome (follow-up)
Pini Prato et al. (2003)	Case series	KTW augmentation	Human autologous fibroblasts from gingiva	DMEM containing FBS and antibiotics (penicillin and streptomycin)	Fully esterified benzyl ester hyaluronic acid (HA)	Autologous fibroblast + HA scaffold (7)	/	KTW gain: 2.0 ± 0.4 (3m)

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/JPER.19-0353.

McGuire & Nunn (2005)	Split-mouth RCT	KTW augmentation	Human allogenic fibroblasts from newborn foreskin	NA	Bioabsorbable polyglactin mesh	HF-DDS (25)	FGG (25)	KTW gain test group: 1.26 mm KTW gain control group: 2.57 mm Test group: higher

graft shrinkage, better color and texture match with surrounding tissues (1y)

Mohammadi et al. (2007)	Split-mouth RCT	KTW augmentation	Human autogenous fibroblasts from attached gingiva	Nutritional medium containing AB human serum and antibiotics (penicillin and streptomycin)	CM	CGG (9)	Periostealfenestrationtechnique (9)	KTW gain test group: 2.8 mm KTW gain control group: 1.9 mm AGW gain test group: 2.8

								mm AGW gain control group: 2 mm (3m)
McGuire et al. (2008)	Split-mouth RCT	KTW augmentation	Human allogenic fibroblasts and keratinocytes from foreskin	NA	CM	BCT (25)	FGG (25)	KTW gain test group: 2.4 ± 1.0 mm KTW

gai
n
con
trol
gro
up:
4.5
±
0.8
mm

Tes
t
gro
up:
bet
ter
col
or
and
text
ure
mat
ch
wit
h
surr
oun

								ding tissues, greater patient preference (6m)
Nevins (2010)	Case series	KTW augmentation	Human allogenic fibroblasts and Keratinocytes from foreskin	Agarose-rich nutrient medium	CM	LCC (5)	/	All cases healed with histologically normal gin

giva mu cos a and sho we d incr eas ed KT W. Tiss ue col or and text ure mat che d well the adj ace nt

								tissue
McGuire et al. (2011)	Split-mouth RCT	KTW augmentation	Human allogenic Keratinocytes and fibroblasts from foreskin	Agarose-rich nutrient medium	CM	LCC (85)	FGG (85)	KTW gain test group: 3.2 ± 1.1 mm KTW gain control group: 4.5

7 ± 1 mm AGW gain test group: 1.7 ± 1.3 mm AGW gain control group: 3.2 ± 1.2 mm (6m

								)
Morelli et al. (2011)	Split-mouth RCT	Angiogenic-related biomarkers expression during the early wound healing	Human allogenic Keratinocytes and fibroblasts from foreskin	Agarose-rich nutrient medium	CM	LCC (44)	FGG (44)	No correlation between the investigated angiogenic biomarkers and

wo
und
-
hea
ling
sco
res.
By
we
ek
1,
VE
GF,
IL-
8,
FGF
-2,
PD
GF-
BB
and
ang
iost
atin
wer
e
sign
ifica

ntly up-regulated in the test group compared with control group

Izumi et al. (2013)	Case series	KTW augmentation	Human autogenous keratinocytes from hard palate	Serum-free medium containing calcium, pituitary extract and antibiotics (gentamicin and amphotericin B)	ADM	EVPOME (5)	/	KTW gain 3.0 mm (6m)
Scheyer et al. (2014)	Post-hoc analysis of data collected in two previous studies [1, 2]	Evaluation of changes following KTW augmentation	Human allogenic Keratinocytes and fibroblasts from foreskin	NA	CM	LCC (110)	FGG (110)	LCS resulted in more site-appropriate tissue than FGG reg

ardi
ng
col
or
mat
ch
wit
h
surr
oun
din
g
tiss
ues,
abs
enc
e of
scar
/kel
oid
for
mat
ion
and
MG
J
alig
nm

								ent
Wilson et al. (2005)	Split-mouth RCT	Root coverage	Human allogenic fibroblasts from newborn foreskin	NA	Bioabsorbable polyglactin mesh	HF-DDS (13)	CTG (13)	mRC test group: 56.7 ± 27.8 % mRC control group: 64.4 ± 31.9 % KT

								W gain test group: 0.2 mm KT W gain control group: 0.2 mm (6,)
Murata et al. (2008)	Case series	Root coverage	Human autologous fibroblasts form the retro molar region of the mandible	DMEM containing FBS	Two layered matrix of hyaluronic acid sponge and atelo-collagen gel	CGDS + scaffold (14)	/	mRC: 79.1 ± 25.7 % KT

								W gain: 1.1 ± 1 mm AG W gain: 1.5 ± 1.1 mm (30.7±9.6w)
Jhaveri et al. (2009)	Split-mouth RCT	Root coverage	Human autogenous fibroblasts from attached gingiva	α-MEM containing FBS and antibiotics (penicillin and streptomycin)	ADM	ADM + F (10)	CTG (10)	mRC test group: 83.3 ± 27.

	2%
mRC control group: 83.3 ± 27.2%	
KTW gain test group: 0.5 mm	
KTW gain control	

								group: 0.8 mm (6m)
Köseoğlu et al. (2013)	Split-mouth RCT	Root coverage	Human autogenous fibroblasts from palatal mucosa	DMEM containing FBS and antibiotics (penicillin and streptomycin)	CM	CM + F (11)	CM (11)	mRC test group: 69.6 ± 29.3% mRC control group: 38.3 ± 32.6% KT

	W gain test group: -0.41 mm
	KT W gain control group: -0.36 mm (1y)

Milinkovic et al. (2014)	Split-mouth RCT	Root coverage	Human autogenous fibroblasts from the hard palate	Nutritional medium	CM	AFCC + CM (18)	CTG (18)	mRC test group: 89.9% mRC control group: 91.3% KTW gain test group: 1.74 mm KT

								W gain control group: 2.26 mm Similar RES between the two groups (1y)
Zanwar et al. (2014)	Parallel RCT	Root coverage	Human allogenic stem cells from umbilical cord	Serum-free medium specifically formulated for stem cells	PLA/PGA	PLA/PGA + stem cells (12)	CTG (12)	mRC test group: 72.

	4 ± 13.6 %
mRC control group:	82.1 ± 11.0 %
KTW gain test group:	0.9 ± 0.7 mm
KTW	

								gain control group: 1.4 ± 0.8 mm (6m)
Zanwar et al. (2017)	Parallel RCT	Root coverage	Human allogenic stem cells from umbilical cord	Serum-free medium specifically formulated for stem cells	PLA/PGA	PLA/PGA + stem cells (7)	PLA/PGA alone (7)	mRC test group: 66.3 ± 27.0 % mRC control group:

	57.4 ± 15.6 %
KT W gain test group:	0.7 ± 0.8 mm
KT W gain control group:	0.8 ± 0.6 mm

(6m
)

Legend. EVPOME: Ex vivo produced oral mucosal equivalent; KTW: Keratinized tissue width; ADM: Acellular Dermal Matrix; RCT: Randomized Control Clinical Trial; α-MEM: α- minimal essential medium; FBS: fetal bovine serum; F: Fibroblasts; CTG: Connective tissue graft; mRC: mean root coverage; DMEM: Dulbecco modified Eagle medium; CM: Collagen matrix; NA: Not available; HF-DDS: Human fibroblasts derived dermal substitute; FGG: Free gingival graft; BCT: Bilayered cell therapy; LCC: Living cellular construct; AGW: Attached gingiva width; AFCC: Autologous fibroblast cell culture; CGG: Cultured gingival graft; CGDS: Cultured gingival dermal substitute; LCT: bilayered live cell therapy; LCS: Living cellular sheet; PLA/PGA: polylactic acid/polyglycolic acid

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/JPER.19-0353.

Figure Legend

Figure 1. Soft tissue augmentation using EVPOME. **A)** Schematic drawing illustrating the composition of the EVPOME where oral keratinocytes are seeded within a cell culture media with the necessary signaling molecules to push their development along the desired path. These cells are then cultivated on ADM that serves as scaffold for developing a full-thickness TEC; **B)** Four days submerged in culture after seeding of oral keratinocytes (day 4); **C)** EVPOME raised to an air-liquid interface; **D)** EVPOME grown at an air-liquid interface for 7 days (day 11); **E)** EVPOME grown for additional 7 days (day 18) showing increased cell stratification (Adapted with permission from Journal of Dental Research[13] and from International Journal of Oral Maxillofacial Implants[16])

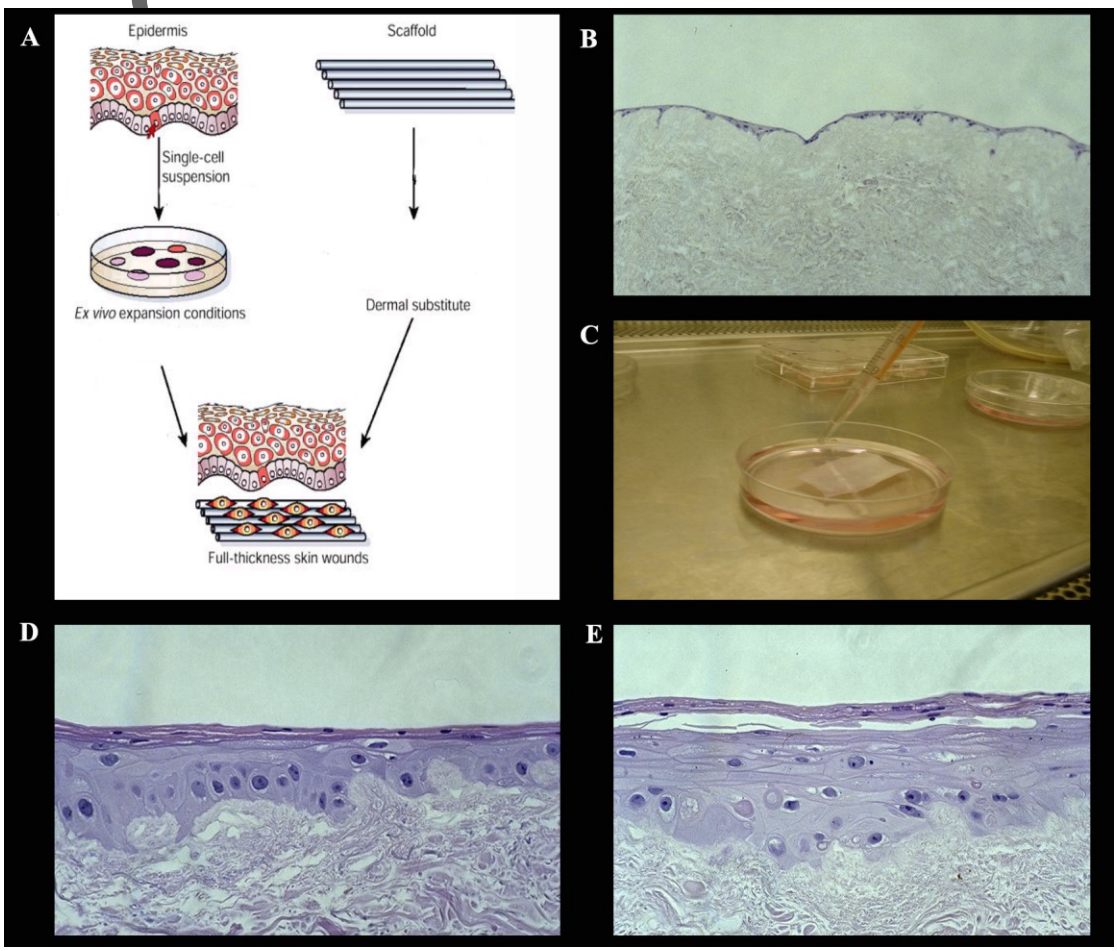

Figure 2. Soft tissue augmentation using EVPOME. **A)** Baseline clinical scenario showing the limited band of keratinized tissue in the anterior mandible area; **B)** The EVPOME construct immediately before grafting; **C)** The EVPOME sutured over the periosteum with interrupted sutures. The TEC was then covered by a periodontal dressing; **D)** Outcomes at 30 days. (Adapted with permission from International Journal of Oral Maxillofacial Implants[16])

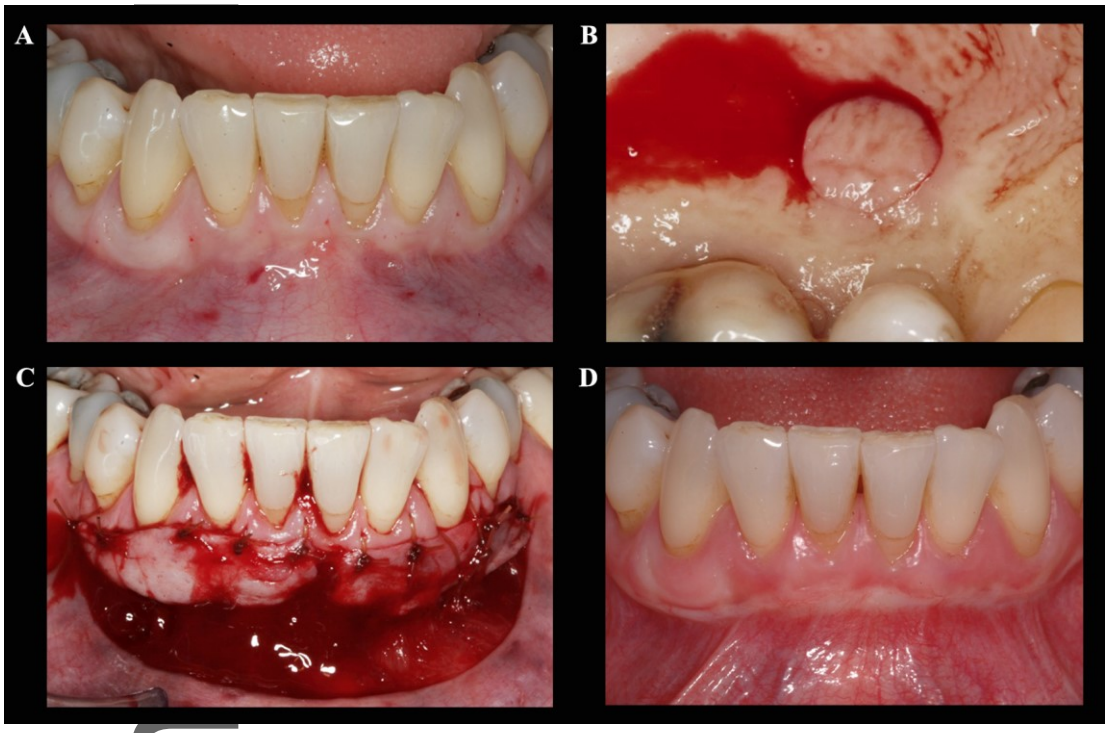

Figure 3. Efficacy of the Living human fibroblast-derived dermal substitute (HF-DDS) in increasing keratinized tissue width **(A-D)** and in the treatment of gingival recessions **(E-F)**. **A)** Pre-operative situation showing the limited keratinized tissue around the lower right premolars; **B-C)** After preparation of the receiving bed, the HF-DDS was positioned and sutured to the papilla regions; **D)** Healing at 1 year showing the keratinized tissue width gain; **E)** Gingival recession in the maxillary right lateral incisor and canine; **F)** 6-month outcomes after coronally advanced flap + HF-DDS showing complete root coverage. (Adapted with permission from Journal of Periodontology [41])

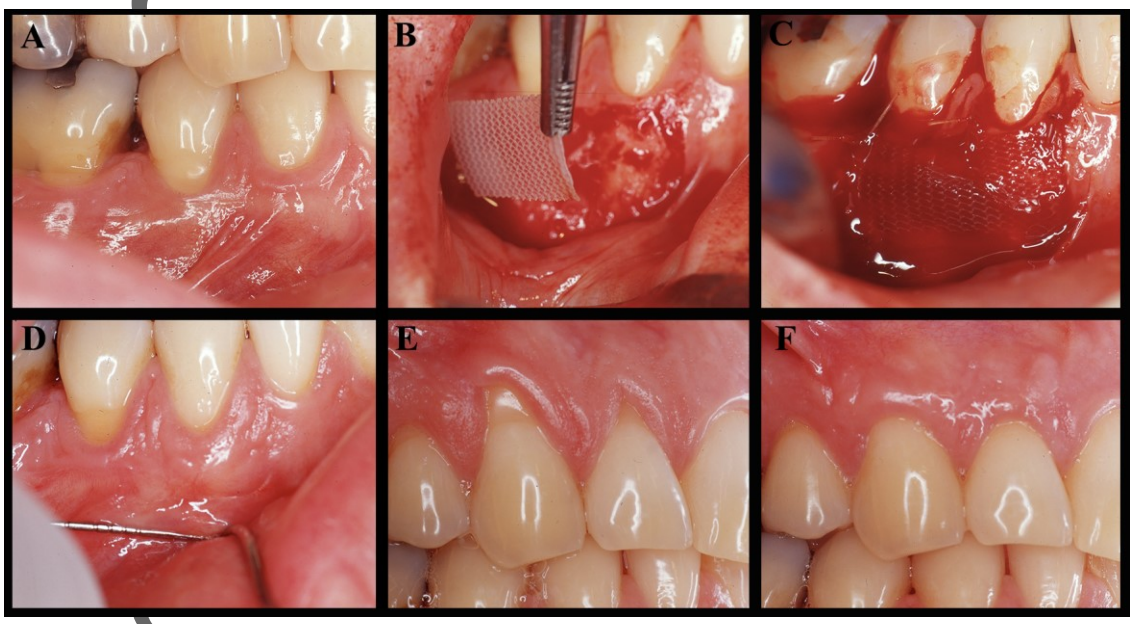

Figure 4. Efficacy of the Living cellular construct (LCC) in increasing keratinized tissue width. **A)** Pre-operative situation showing limited keratinized tissue around a mandibular right premolar; **B)** After preparation of the recipient bed, the LCC was firmly sutured over the periosteum; **C)** 6-month outcomes showing increased keratinized tissue and excellent esthetic results.