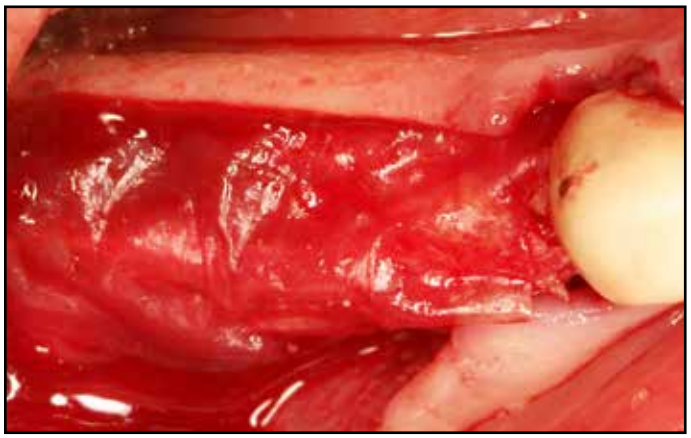
An Updated Primer on the Utilization of Amnion-Chorion Allografts in Dental Procedures

Dan Holtzclaw, DDS, MS[1] • Robert Tofe, MBA[2]

Abstract



While placentally derived allografts have been utilized in medical procedures for over 100 years, their use in dental procedures is relatively new with only 10 years of continuous history. As in medical procedures, the initial applications of placental grafts in dental procedures were with amnion products. More recently, however, advanced placental allografts such as laminated amnion-chorion products have displaced the use of amnion-only products. The addition of a chorion layer to amnion allograft has produced a number of improvements over amnion alone including increased thickness of the membrane and a 20-fold increase in growth factor content. The goal of this paper is to provide an updated primer on the utilization of dehydrated human amnion-chorion membrane (dHACM) allografts in dental procedures. The science behind this material is reviewed along with an examination of current and future dental uses.

KEY WORDS: Amnion, chorion, allograft, wound healing, review

1. Private practice limited to dental implants and oral reconstructive surgery. Austin, Texas, USA

2. Snoasis Medical, Inc. Denver, Colorado, USA

INTRODUCTION

Placentally derived products have been used as wound healing adjuncts for more than a century in numerous medical applications. In the early 1900's, placentally derived human amnion was utilized for skin transplantations as reported by Davis[1] in a review of 550 treated cases. By the 1940's amnion was routinely applied in a variety of ophthalmologic surgeries with documentation of faster healing and improved outcomes.[2,3] By the 1950's and 1960's amnion was being utilized in ENT procedures such as mastoidectomy[4] and myringoplasty.[5] More recently, placentally derived tissues such as amnion and amnion-chorion have been used in the treatment of diabetic ulcers,[6,7] Moh's micrographic surgery,[8] free flap surgical treatment of venous insufficiency/lymphedema,[9] vaginal reconstructive surgery,[10,11] and as an adjunct to protect neurovascular bundles during prostate surgery,[12] among others. With the successful and well documented medical applications of placentally derived amnion and amnion-chorion tissues, the crossover of these products into the realm of dental treatment is not surprising. In 2008, a multi-layered dehydrated human amnion membrane (dHAM) (BioCover™, Snoasis Medical, Denver, Colorado, USA) was introduced to the dental market as a potential treatment for gingival recession. By 2010, dehydrated human allograft composed of laminated amnion-chorion (dHAMC) (BioXclude™, Snoasis Medical, Denver, Colorado, USA) expanded the potential applications of placentally derived products to a much wider variety of dental procedures. As placentally derived products are still relatively new to the field of dentistry, the purpose of this paper is to review the general characteristics of contemporary amnion-chorion products and their current applications for dental procedures.

Figure 1: BioXclude™ dHACM (arrow) averages ~300µm in cross-sectional thickness.

Basic Placental Tissue/Amniotic Sac Primer

During pregnancy, membranes comprising the amniotic sac contain the developing embryo and fetus. The innermost membrane of the amniotic sac, the amnion, encloses the fetus and amniotic fluid, while the outer layer, or chorion, contains the amnion and interdigitates with the maternal decidual tissues to form the placenta. The amnion and chorion contain no blood vessels, have no direct blood supply, and receive nutrients by diffusion from the amniotic fluid and maternal decidua.[13,14] Amnion contains several extracellular matrix (ECM) proteins, including fibronectin, laminins, proteoglycans, glycoproteins, and collagen types I, III, IV, V, and VI.[14-16] The chorionic tissue can be up to 4 times thicker than the amnion[17] and, similar to the amnion, is composed of fibronectin, laminins, and collagens I, III, IV, V, and VI.[16] A particularly unique feature of the membranes composing the amniotic sac is their role in protecting the developing fetus from the maternal immune system. Although it is not fully understood why, the maternal immune system accepts the develop-

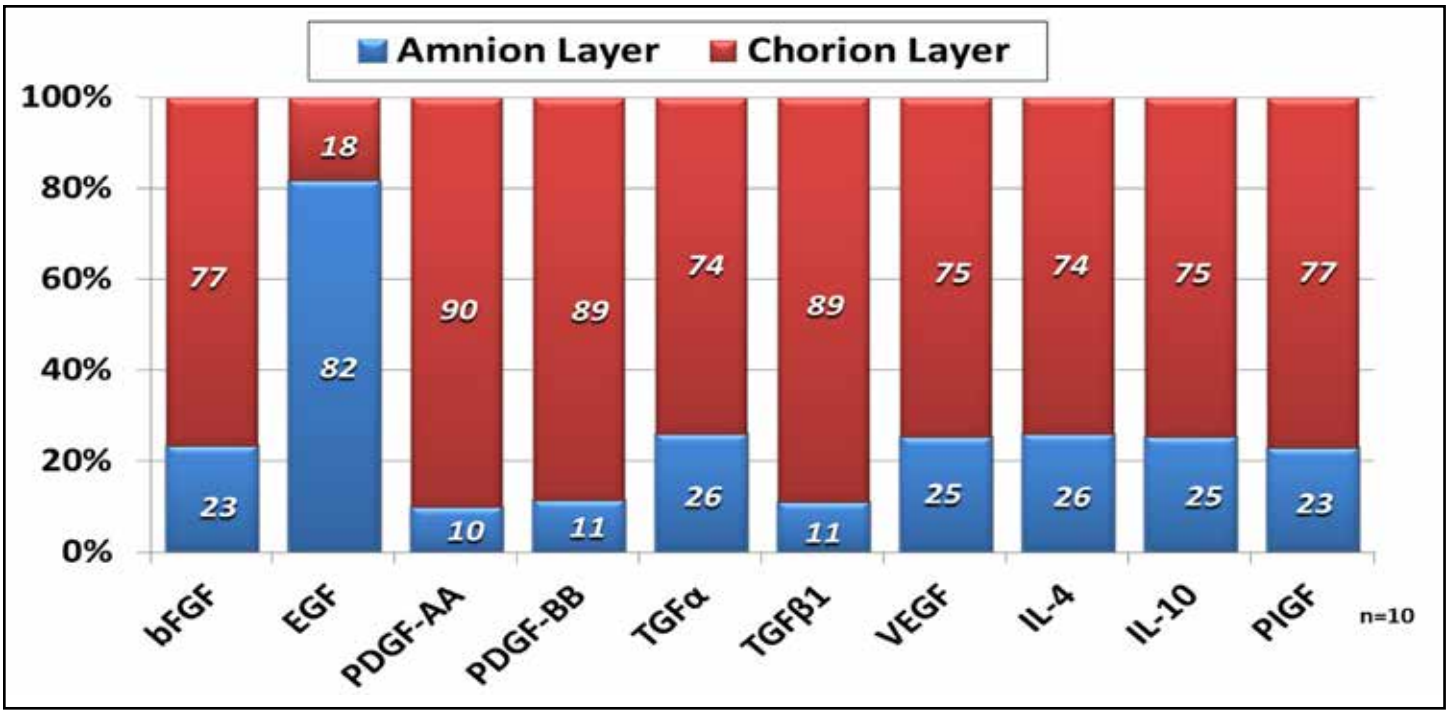


Figure 2: Total growth factor content increases dramatically with the addition of chorion to amnion.

ing fetus in spite of its foreign nature. It has been postulated that immunoregulation may occur at the fetal-maternal interface which inhibits maternal T-cell,[18] lymphocyte,[19] and natural killer cell[20] proliferation. This function has bestowed the term *immunopriviledged* upon tissues including the amnion and chorion meaning they elicit little to no immunological response in foreign hosts.[21,22]

Amnion-Chorion Allograft Production

Placental tissue used for the production of BioXclude™ dental amnion-chorion allograft is obtained from consenting mothers delivering full-term babies via elective cesarean section surgery, as regulated by the Food and Drug Administration (FDA) and American Association of Tissue Banks (AATB).[23] All donors are screened for infectious diseases, including but limited to, human immunodeficiency virus (HIV) type 1 and 2 antibodies, HIV type 1 nucleic acid test, human T-lymphotropic virus (HTLV) type 1 and 2 antibodies, hepatitis B surface antigen, hepatitis B core total antibody, hepatitis C antibody, hepatitis C virus nucleic acid test, and syphilis.[24,25] Upon collection of the placental tissues, they are placed in quarantine storage until clean serology reports are confirmed. Upon acceptable serology confirmation, the amnion and chorion are isolated and prepared with the proprietary Purion® process (MiMedx, Marietta, Georgia, USA). The Purion® process was developed in 2006 as a method for gently cleansing and dehydrating amniotic membrane allografts while preserving the structural integrity and biochemical activity of the tissue. The Purion® process is used to produce dehydrated human amnion-chorion membrane (dHACM) whereby de-epithelialized placental amnion is laminated to chorion tissue that is sourced directly

Table 1: Examples of Growth Factors, Cytokines, Chemokines, etc. Found in dHACM30					
GCSF	GM-CSF	GDF-15	IFNγ	IL-1α	IL-1β
IL-1Ra	IL-4	IL-5	Ang	Ang-2	bFGF
BMP-5	BDNF	EG-VEGF	EGF	FGF-4	KGF
FGF-7	IL-6	IL-7	IL-10	IL-12p40	IL-12p70
IL-15	IL-17	MCSF	OPG	BLC	Eotaxin-2
I-309	IL-8	IL-16	MCP-1	MIG	MIP-1α
MIP-1β	MIP-1d	RANTES	GH	HB-EGF	HGF
IGF-1	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-6
B-NGF	PlGF	PDGF-AA	PDGF-BB	TGF-α	TGF-β1
VEGF	TIMP-1	TIMP-2	TIMP-4	6Ckine	ADAMTS13
APRIL	aFGF	Activin-A	Adiponectin	Adipsin	AgRP
ANG-1	ANG-4	ANGPTL3	ANGPTL4	Angiostatin	ACE-2
BAFF	BTC	BMP-	2BMP-7	BMP-9	CRP
CXCL14	CXCL16	CA9	CEA	Chemerin	CNTF
Ckβ8-1	Clusterin	CF XIV	C5a	Cripto-1	Cystatin A
Cystatin B	Cystatin C	Cystatin EM	DAN	DcR3	DLL1
DKK-1	DKK-3	DKK-4	Eotaxin	Eotaxin-3	ENA-78
FABP2	Fetuin A	FGF-6	FGF-9	FGF-19	FGF-21
Flt-3L	FSH	Follistatin	FLRG	Fractalkine	Furin
GASP-1	GASP-2	Galectin-1	Galectin-2	Galectin-3	Galectin-7
Galectin-9	GDNF	gp130	GCP-2	Granulysin	Gas1
GROα	GRO	HCC-1	HAI-2	hCGβ	Insulin
IGF-2	IGFBP-5	IP-10	I-TAC	IL-1 F5	IL-1 F6
IL-1 F7	IL-1 F8	IL-1 F9	IL-1 F10	ST2	IL-2
IL-3	IL-6sR	IL-8	IL-11	IL-17B	IL-17C
IL-17E	IL-20	IL-21	IL-23	IL-24	IL-27
IL-32α	IL-33	IL-34	Kallikrein-5	Kallikrein-14	LAP(TFGb1)
Legumain	Leptin	LRIG3	Liocalin-2	Limphotactin	MIF
MBL	Marapsin	Midkine	MCP-2	NOV	NSE
NT-3	NT-4	NAP-2	OSM	Osteoactivin	OPN

from the amniotic sac.[26] Lamination of the amnion and chorion produces a graft that is significantly thicker (~300µm)[27] (Figure 1) compared to layered amnion alone (~<100µm).[28] Processed dHACM are terminally sterilized by gamma irradiation prior to sterile packaging, which in addition to increasing the safety of the product, has proven not to affect the bioactivity of the allograft.[29]

Biologic and Immunoregulatory Properties of dHACM

In testing the effects of the Purion® process on the bioactivity of dHACM allografts, 226 regulators of healing and inflammation were identified in the processed grafts.[30] These include tissue promoting growth factors, immunomodulatory cytokines, and immunomodulatory chemokines (Table 1). While it is beyond the scope of this paper to specifically discuss the properties of each of these growth factors, it is well established that these factors aid and promote healing in a variety of capacities which will be discussed in this paper. It is important to note that the thicker graft produced by the lamination of amnion to chorion in the production of dHACM such as BioXclude® results in growth factor content that is up to *twenty times* greater than that which is seen in amnion only allografts (Figure 2).[31] The rich growth factor and immunomodulatory content of dHACM likely plays a role in the anti-inflammatory, antibacterial, pain reduction, angiogenic, and enhanced wound healing properties that have been identified with the product.

Anti-Inflammatory Properties

Amniotic tissue has been shown to reduce inflammation in studies designed specifically to study inflammation. Solomon et al.[32] cultured human corneal limbal epithelial cells on either freshly frozen and thawed human amniotic membrane or tissue culture plastic. These cells were plated on amnion tissue and assayed for the expression of inflammatory cytokines. The cultures demonstrated that cryopreserved amnion directly suppressed the expression of pro-inflammatory cytokines at the protein and mRNA levels. In another study of transepithelial photorefractory keratectomies in rabbits, the application of fresh amnion showed a significant reduction in the number of leukocytes and less keratocyte death compared to controls, demonstrating the anti-inflammatory effects of amnion.[33] When studying the effects of amniotic membrane on corneal wounds in rabbits via histopathologic, proteinase assay, and zymography, Kim and colleagues[34] reported decreased polymorphonuclear leukocyte (PMN) infiltration, decreased macrophage chemotaxis, and inhibited proteinase activity at treated sites. In reviewing the use of amniotic grafts for ocular surface reconstruction, Tseng[35] noted the anti-inflammatory effects of the graft as did Güell et al.[36] in their treatment of symptomatic bullous keratopathy. Koob and colleagues have performed multiple studies evaluating amniotic tissues such as dHACM for anti-inflammatory modulators via enzyme linked immunosorbent assays (ELISA) with significant findings.[37,38]

Anti-Bacterial Properties

A number of studies have demonstrated the antibacterial nature of amniotic tissue.[39,40] Expression of antimicrobial peptides such as βdefensins, elafin and SLPI, which are essential elements of the innate immune system, may be associated with antibacterial properties of amniotic tissue.[39,40] Tehrani and colleagues[39] evaluated the

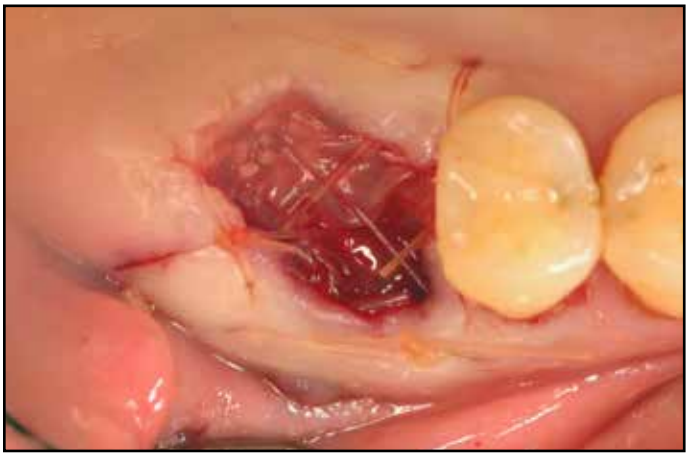


Figure 3a: Extraction site treated with BioXclude™ dHACM at Day 0.

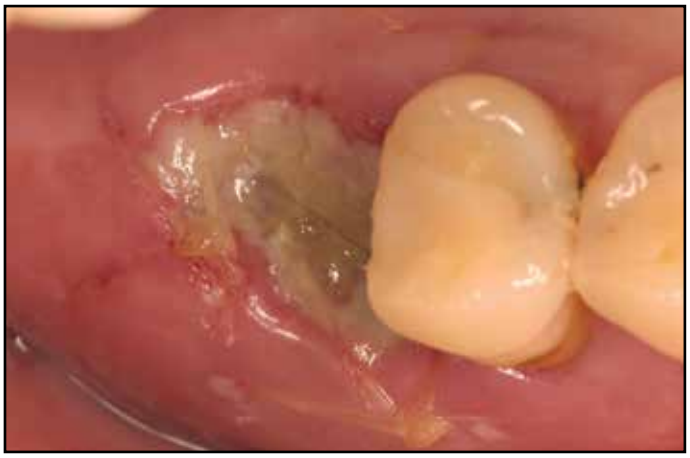


Figure 3b: Extraction site treated with BioXclude™ dHACM at 24 hours.

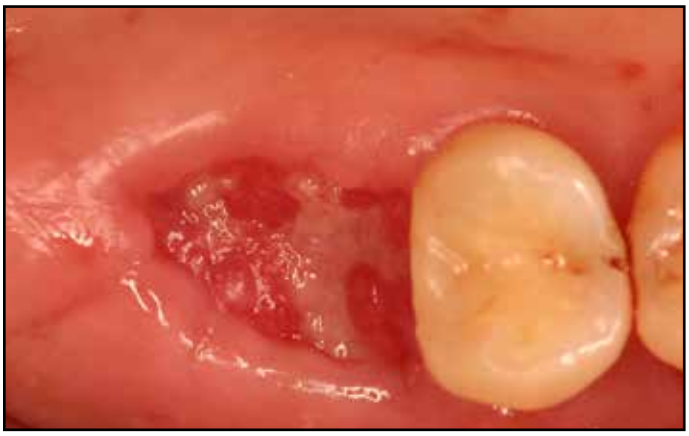


Figure 3c: Extraction site treated with BioXclude™ dHACM at 96 hours. Note migration of tissue from wound edges towards center.

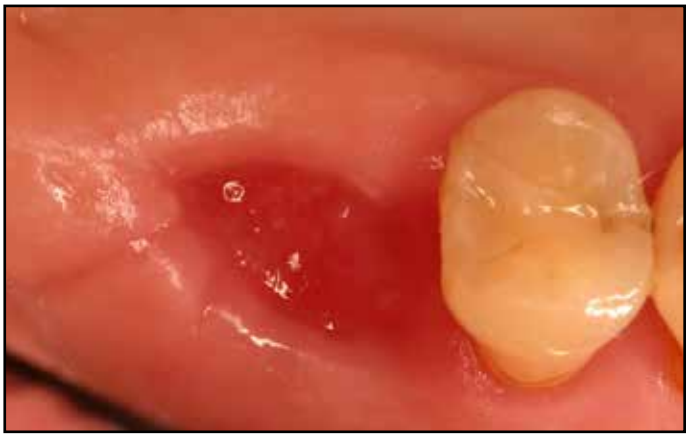


Figure 3d: Extraction site treated with BioXclude™ dHACM at Day 21.

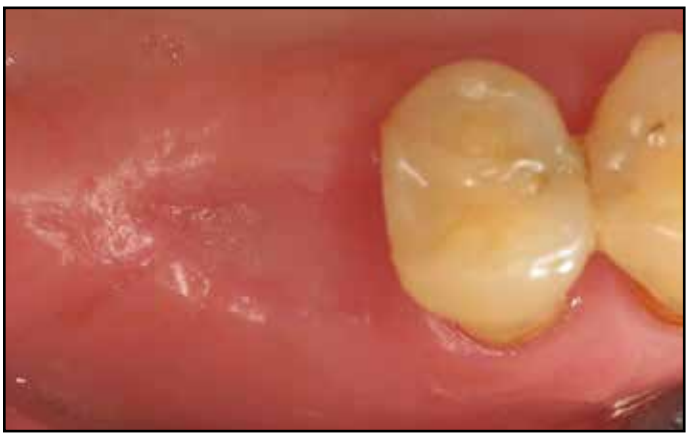


Figure 3e: Extraction site treated with BioXclude™ dHACM at 3 months.

antibacterial properties of cryopreserved and dehydrated amniotic tissue against a variety of bacterial strains including *Staphylococcus aureus ATCC 25923*, *Pseudomonas aeruginosa ATCC 27853*, and *Escherichia coli ATCC 25922*. The results of this study noted that processing of the amniotic tissues did not adversely affect the antibacterial properties of graft. In a separate study, Kjaergaard and colleagues[41] tested the antibacterial properties of chorioamniotic membranes

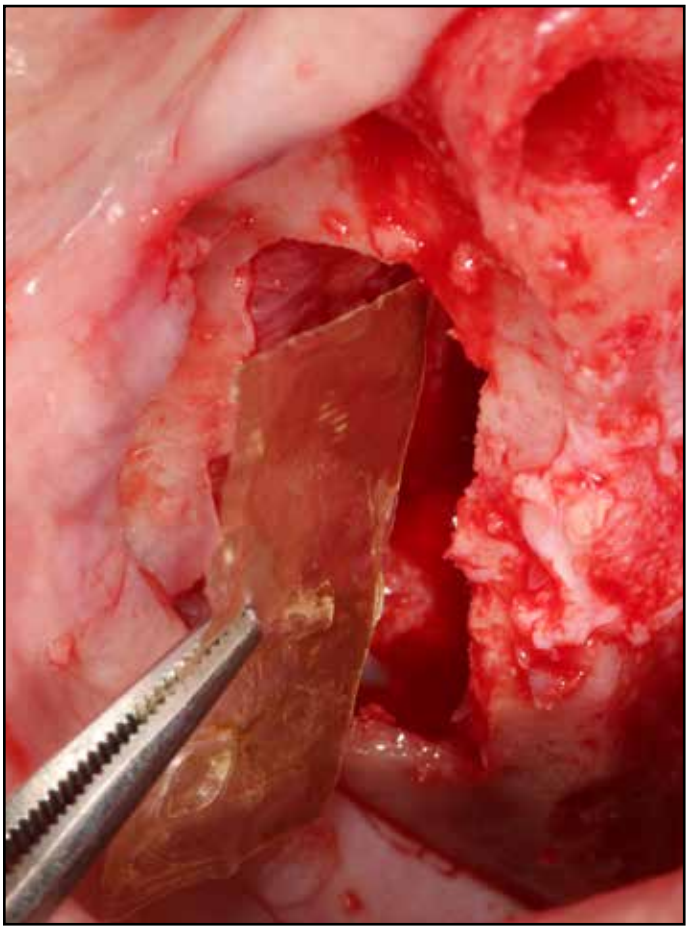


Figure 4a: BioXclude™ dHACM (being placed into the maxillary sinus) is of similar thickness to the Schneiderian membrane.

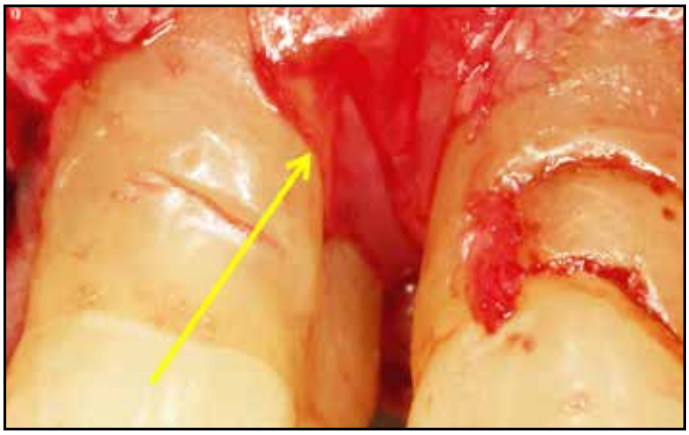


Figure 5: BioXclude™ dHACM may safely touch root surfaces.

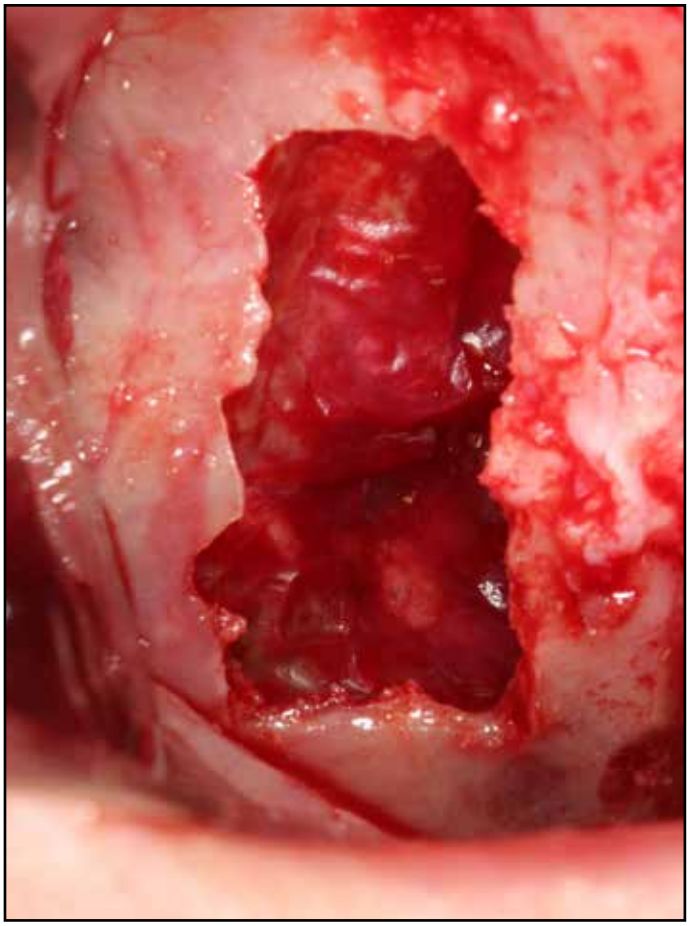


Figure 4b: BioXclude™ dHACM self-adheres to the Schneiderian membrane in the maxillary sinus.

against *Hemolytic streptococci group B* (GBS), *Hemolytic streptococcus group A, Staphylococcus aureus, Staphylococcus saprophyticus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter calcoaceticus* and *Lactobacillus species.* All bacterial strains were inhibited by the amniotic tissues.

Pain Reduction Properties

Numerous studies have noted the pain reduction properties of amniotic grafts when used for a wide variety of applications. In evaluating the treatment of venous leg ulcers, Mermet et al.[42]

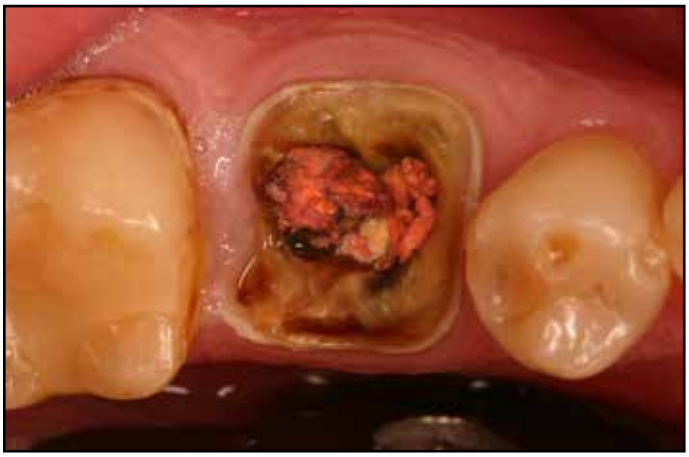


Figure 6a: Mandibular molar prior to extraction.

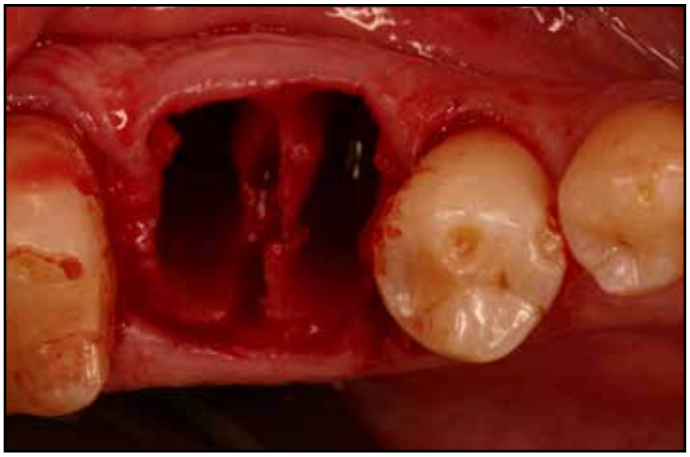


Figure 6b: Mandibular molar following extraction.

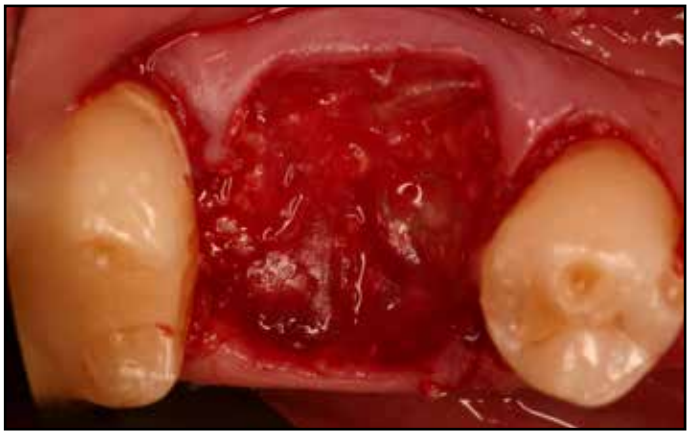


Figure 6c: Mandibular molar extraction site grafted with particulate allograft and covered with BioXclude™ dHACM.

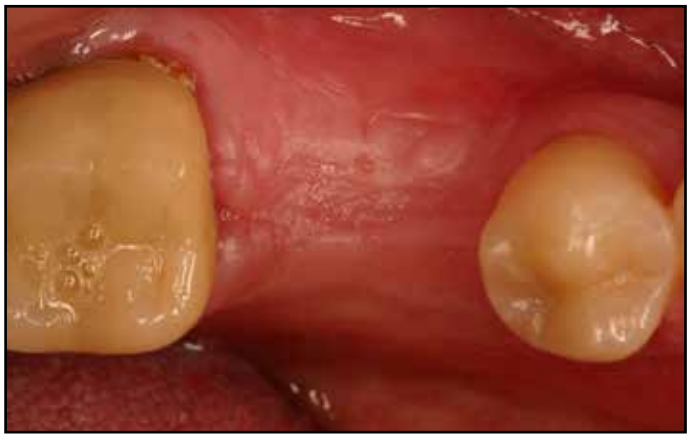


Figure 6d: Gingival tissue healing at mandibular molar site preservation with BioXclude™ dHACM at 3 months healing.

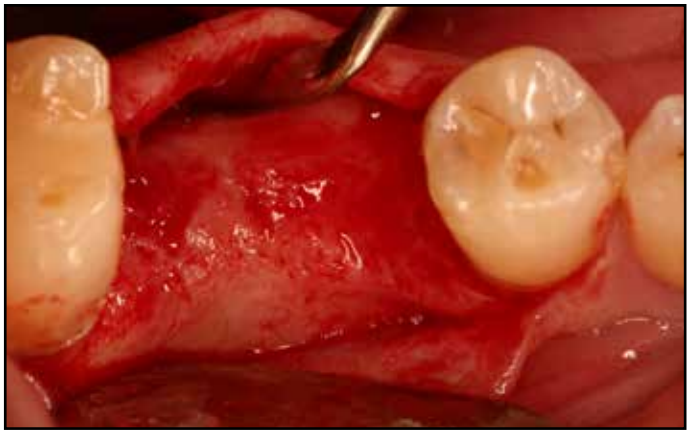


Figure 6e: Bone healing at mandibular molar site preservation with BioXclude™ dHACM at 3 months healing.

noted that all study participants experienced a significant reduction of ulcer-related pain rapidly following amniotic tissue application. In a comparison of vestibuloplasty healing with and without the application of amniotic grafts, Sikkerimath et al.[43] noted increased and longer lasting pain in non-treated patients. In evaluating the use of amniotic tissues as a dressing for skin grafts on burn patients, Eskandarlou and colleagues[44] noted decreased pain with amniotic dressed grafts compared to standard dressings. In evalu-

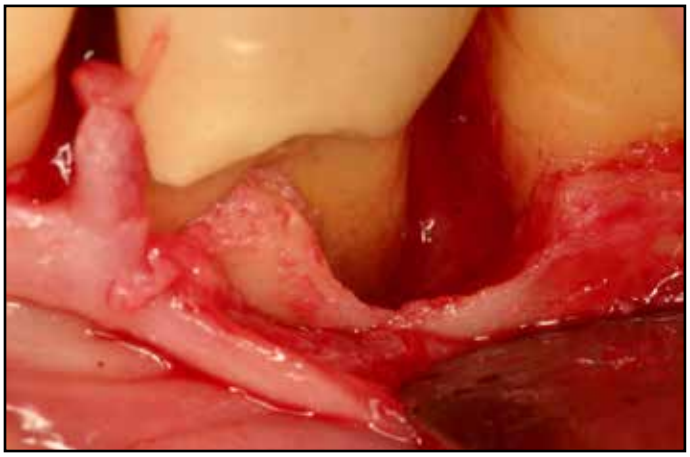


Figure 7a: Intrabony defect at the distal of tooth #19 following hand instrumentation debridement.

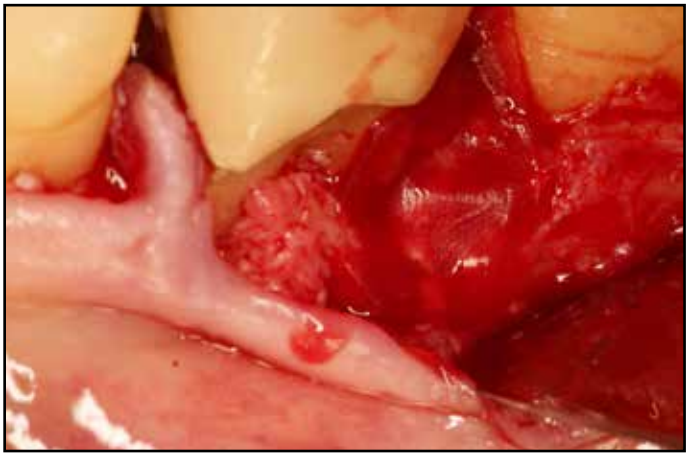


Figure 7b: Intrabony defect at the distal of tooth #19 treated with GTR using particular allograft and BioXclude™ dHACM.

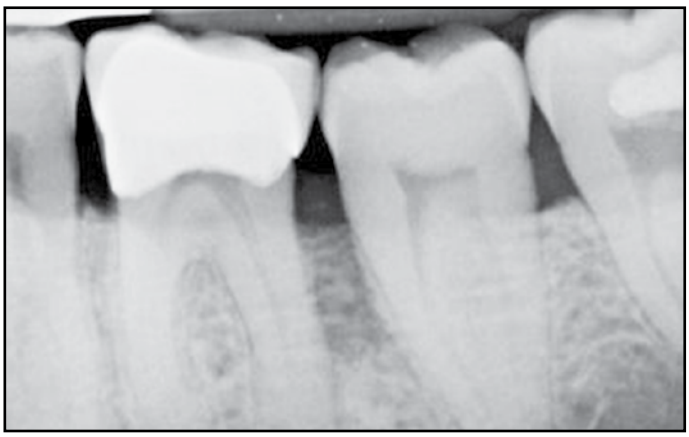


Figure 7c: Radiograph of intrabony defect at the distal of tooth #19 prior to GTR treatment.

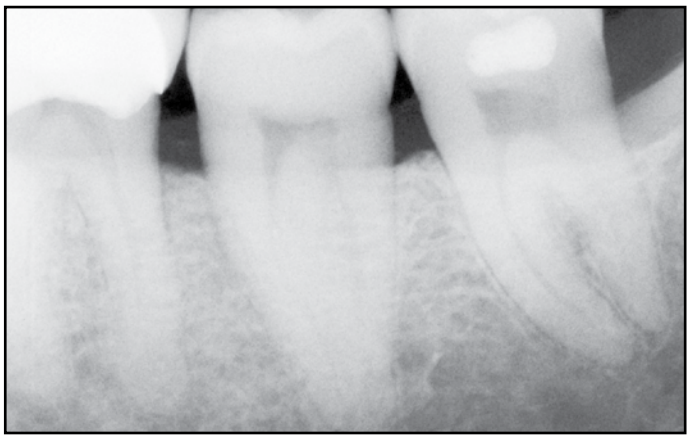


Figure 7d: Radiograph of BioXclude™ dHACM GTR treated intrabony defect at the distal of tooth #19 at 48 months.

ating the use of amniotic tissues in combination with dental implant treatment, Velez et al.[45] noted a significant reduction in pain for treated patients, especially during the first 144 hours. In the author's personal use of dHACM in thousands of dental surgeries, he has likewise noted post-surgical pain reduction when the material is utilized.

Angiogenic Properties

Angiogenic properties of chorioamniotic membranes were recognized and documented in medical literature as far back as the 1980's.[46,47] These findings were expanded upon over the next 30 years[48-51] with findings of angiogenic factors such as endothelins, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF1). More recently, Koob et al.[52] identified angiogenic growth factors in dHACM via ELISA, examining the effects of dHACM extract on human microvascular endo-

thelial cell proliferation and production of angiogenic growth factors. The findings of this study indicated that dHACM grafts contained a multitude of angiogenic factors, even after processing, and that dHACM grafts promoted amplification of angiogenic stimulation via induction of endothelial cell proliferation/migration. Furthermore, this study also found that dHACM grafts upregulated production of endogenous angiogenic growth factors from surrounding endothelial cells and supported the formation of blood vessels in vivo.

Enhanced Wound Healing Properties

Enhanced wound healing has been noted with amniotic grafts for more than a century on a variety of different medical procedures.[1-12] While these earlier studies noted improved healing of patients treated with placentally derived grafts, the exact reasons for this improved healing was a matter of speculation. The aforementioned discoveries of the multitude of growth and Immunoregulatory factors[30] contained in chorioamniotic membranes has provided evidence of the driving force behind the healing capacity of these grafts. The immense regenerative potential of chorioamniotic grafts has recently been tapped for treatment of one of the most difficult healing situations in medicine, non-healing chronic diabetic foot ulcerations (DFU). Distal extremity ulcerations of the foot are widely considered to be one of the most significant complications of Diabetes.[53] Complications associated with non-healing DFU's include pain, neuropathy, limitation of mobility, osteomyelitis, and even amputation.[54] In fact, diabetic complications have been noted in up to 70% of all non-traumatic based amputations of lower limbs.[55] Traditional treatment of DFU's , referred to as the "Gold Standard" or "Standard of Care", has involved sharp debridement, infection management, and off-loading.[53] More recently, multiple studies have utilized dHACM in the treatment of DFU and found improved healing with its application.[56-58] In 2016, Zelen et al.[59] compared healing of DFU's in 100 patients over 12 weeks utilizing a variety of treatments. In this prospective, randomized, controlled, parallel group, multi-center clinical trial weekly applications of collagen-alginate dressings ("Standard wound care") were utilized for 35 patients as a control, bioengineered skin substitute graft was utilized weekly for 33 patients, and dHACM was utilized weekly for 32 patients. By 12 weeks, 51% of the standard wound care patients achieved "complete closure" of DFU's compared to 73% with bioengineered skin substitute graft, and 97% with dHACM. Mean healing times for DFU's in this study were 57.4 days for standard wound care, 47.9 days with bioengineered skin substitute graft , and 23.6 days with dHACM. Also in 2016, DiDomenico et al.[60] published a randomized controlled study where patients with non-healing DFU's were treated with either traditional aforementioned "Standard of Care" treatment (SOC) or SOC in combination with chorioamniotic graft application. Twenty patients were randomly assigned to each group and treated for a period of 12 weeks. Endpoint healing evaluation noted that non-healing DFU's "healed completely" in 70% of chorioamniotic graft treated patients versus only 15% complete healing in the SOC treatment group. In 2015, Penny et al.[61] noted similar healing of DFU's in a Case Series of Diabetic patients treated with dHACM grafts. As with the Zelen[59] and DiDomenico[60] studies that noted "complete healing" or "complete closure" of DFU's by 12 weeks of dHACM treatment, Penny

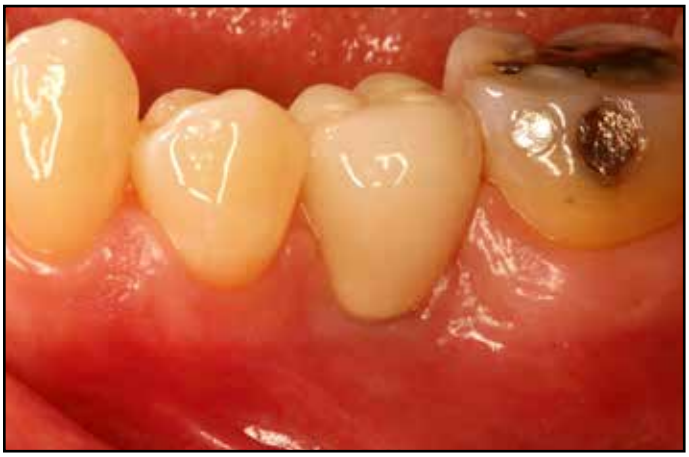


Figure 8a: Intraoral presentation of implant #20 displaying signs of peri-implantitis.

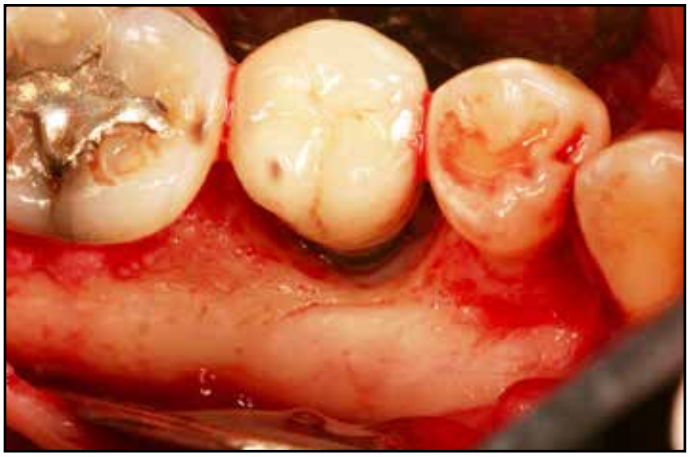


Figure 8b: Lingual view of implant #20 cleansed peri-implantitis intrabony defect.

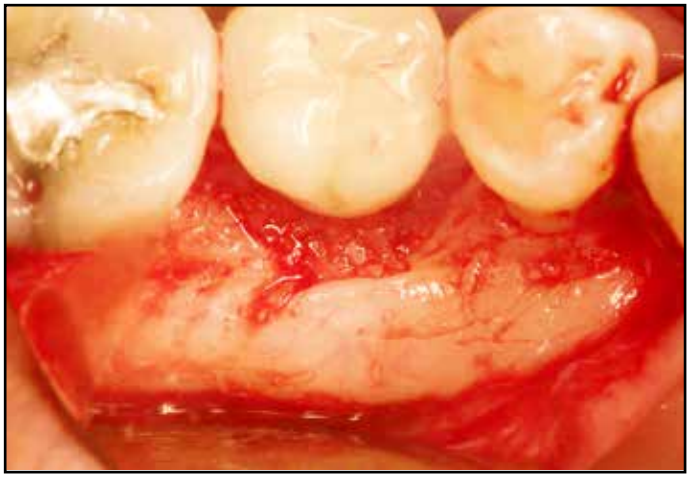


Figure 8c: Lingual view of implant #20 grafted peri-implantitis intrabony defect.

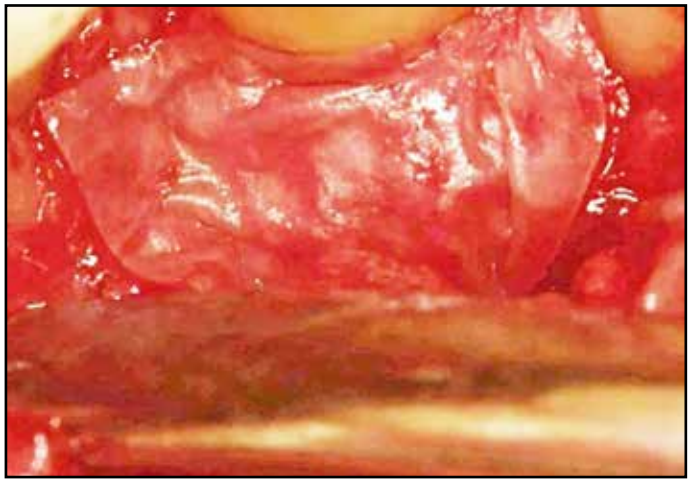


Figure 8d: Lingual view of BioXclude™ dHACM being used for GTR treatment of implant #20 peri-implantitis intrabony defect.

et al. noted healing of dHACM treated DFU's by 11 weeks. These studies, and many others, demonstrate the ability of dHACM to stimulate healing in even the most difficult of environments.

Dental Applications of dHACM

While placentally derived grafts have been used for over a century for medical applications, their use in dentistry is relatively new. The most current form of placentally derived allograft in dentistry is dHACM. Furthermore, the only available dHACM product for dental applications is BioXclude™ (Snoasis Medical, Denver, Colorado, USA). BioXclude™ is a second generation placentally derived product composed of a dehydrated amnion-chorion laminate. Since its introduction in 2010, over 70,000 BioXclude™ dHACM grafts have been utilized for a variety of dental procedures (data

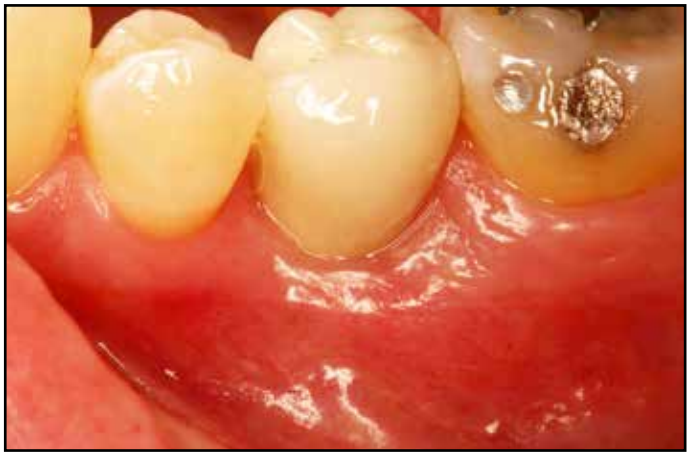


Figure 8e: Intraoral presentation of implant #20 at 24 months after treatment.

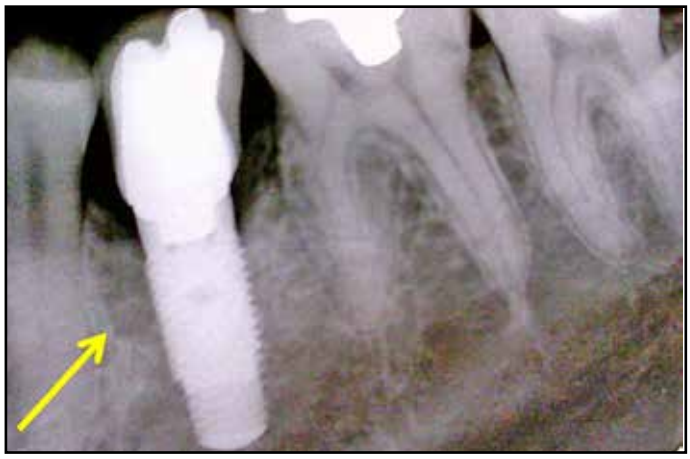


Figure 8f: Pre-surgical radiograph of implant #20 with intrabony defect at mesial (arrow).

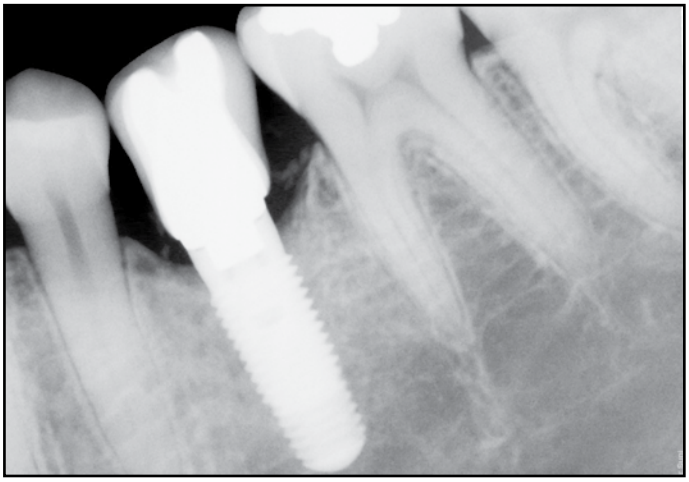


Figure 8g: Post-surgical radiograph of implant #20 at 24 months after treatment.

provided by Snoasis Medical, Denver, Colorado, USA). Research and documentation on the utilization of dHACM for dental procedures has greatly expanded over the past ten years. While the first portion of this paper focused on the historical medical use of this product and the science behind its effectiveness in these applications, the remainder of this paper will focus on the current and future uses of dHACM in dental procedures.

Unique Properties of dHACM in Dentistry

The use of dental dHACM allografts such as BioXclude™ in lieu of traditional dental barrier membranes presents a number of new possibilities that are not readily available with currently available products. First and foremost, unlike traditional dental barrier membranes which may be compromised when left exposed to the oral environment,[62-64] BioXclude™ may be left exposed without reduced healing capacity due to bacterial penetration.[27,65] It is likely that the multitude of growth and immunomodulatory proteins inherently retained in dHACM aid in the rapid epithelial covering of exposed surgical sites in these studies (Figures 3a-f).[3,27,30,37,65] A second unique property of BioXclude™ compared to traditional dental barriers that that dHACM is extremely thin, averaging 300µm in cross sectional thickness whereas collagen barrier membranes average 700-800µm in thickness.[27,66] This makes handling, application, and mucogingival flap adaptation around dHACM much easier than traditional dental membranes.[27] Unlike most barrier membranes, dHACM is self-adhering[66] and does not

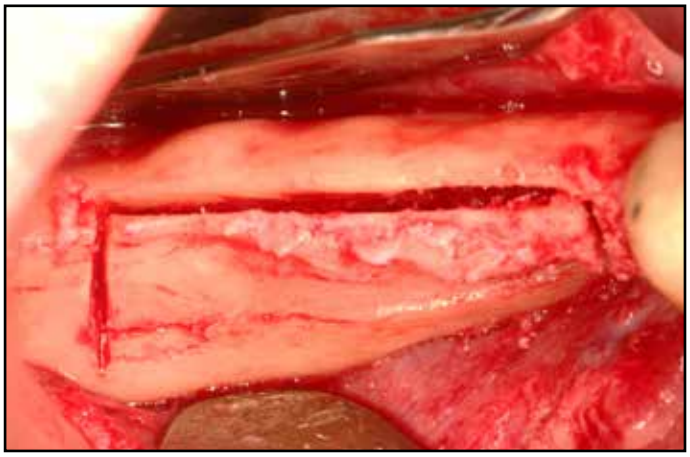


Figure 9a: Ridge split of deficient posterior mandibular ridge for guided bone regeneration.

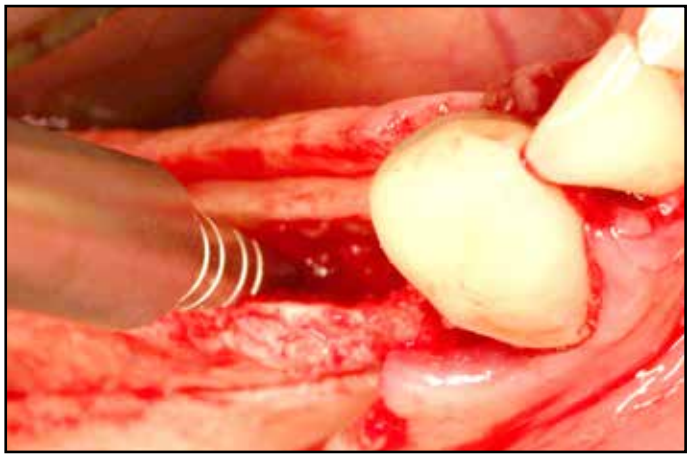


Figure 9b: Ridge splitting osteotome being utilized to expand mandibular ridge.

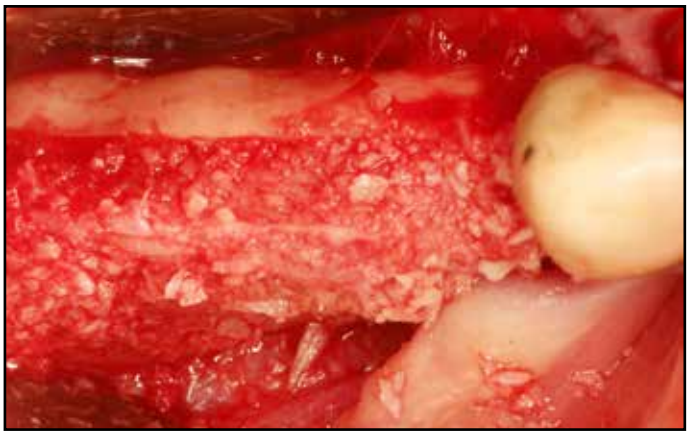


Figure 9c: Expanded mandibular ridge grafted with particular allograft.

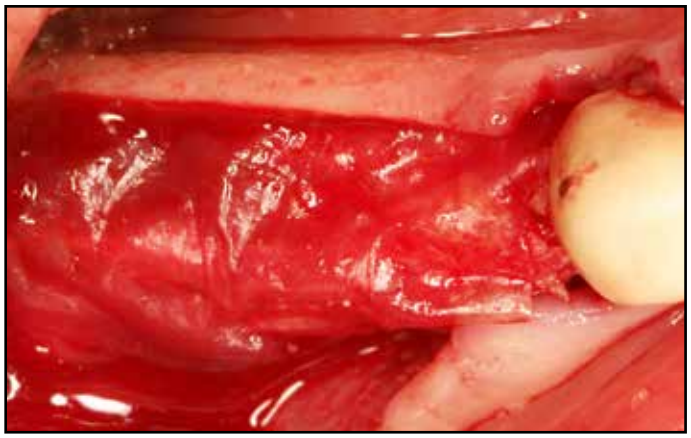


Figure 9d: BioXclude™ dHACM being used for guided bone regeneration treatment of the grafted split ridge.

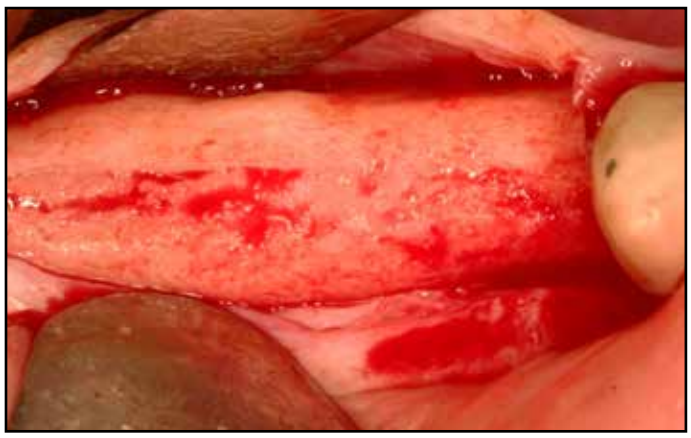


Figure 9e: Healing of BioXclude™ dHACM guided bone regeneration site at 3 months healing.

require sutures for fixation. This feature proves extremely beneficial in delicate situations such as the repair of perforated maxillary sinus membranes (Figures 4a,b).[68,69] Furthermore, unlike traditional dental barriers, dHACM BioXclude™ does not require precise trimming, may touch root surfaces (Figure 5), and may fold upon itself without issue.[68] Finally, dHACM is one of the only known dental membranes that inherently contains a multitude of growth factors[30] and has demonstrated intense immunohistochemical staining for these

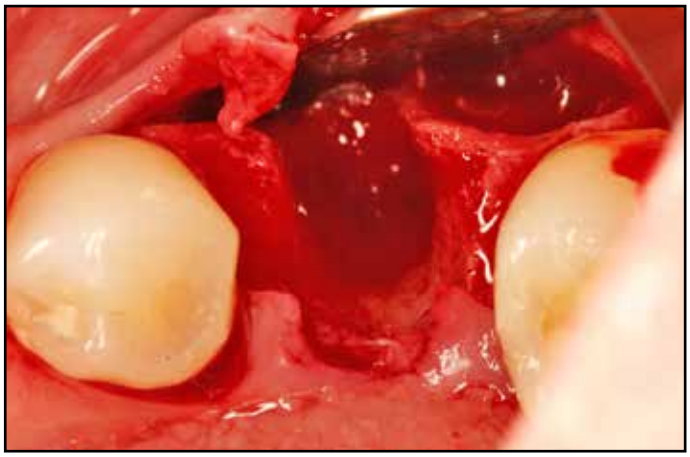


Figure 10a: Buccal dehiscence defect prior to guided bone regeneration treatment with BioXclude™ dHACM.

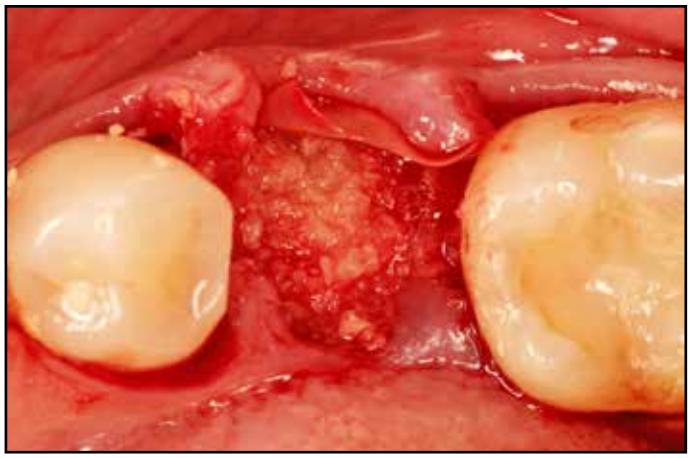


Figure 10b: Walls of the buccal dehiscence defect support the overlying BioXclude™ dHACM.

factors compared to virtually none for traditional dental barriers.[70] The wide variety of properties unique to BioXclude™ avail the product for use in a number of different dental procedures.

Extraction Site Preservation

One of the most common applications for BioXclude™ dHACM is for site preservation following the extraction of teeth (Figures 6a-e). The earliest known study in which BioXclude™ dHACM was used for extraction site preservation was released by Holtzclaw et al. in 2011.[71] In this 5 patient case series, extraction site preservation was performed with BioXclude™ dHACM. This particular study differed from most previously published extraction site preservation studies in the fact that the barrier was left fully exposed to the oral environment with intentional non-primary closure. Average healing times of 3 months were given prior to the placement of dental implants. At this time, all sites had healed with keratinized gingival tissue and had sufficient bone growth to allow the placement of dental implants. After nearly 6 years of loading, dental implant survival rate in this Case Series remains at 100%.

A second site preservation study was later released by Wallace and Cobb in 2011.[72] In this study, 7 patients had extraction site preservation performed with BioXclude™ dHACM and freeze dried bone allograft. Unlike the earlier Holtzclaw study of 2011, the surgical sites in this particular study all received primary closure. After an average healing period of 13 weeks, trephine core sampling and dental implant placements were performed. Histological analysis revealed no residual dHACM and 54.5% new bone formation.

In a 2014 case series study, Holtzclaw[65] performed extraction site preservation procedures on 10 consecutive patients whereby teeth were removed and bone graft was placed and covered with a single layer of BioXclude™ dHACM. The grafted extraction sites had non-primary closure with the amnion-chorion barriers left fully exposed to the oral environment. After an average of 14.2 weeks of healing, all BioXclude™ treated extraction sites had complete gingival closure. Trephine bone core samples obtained at this time revealed a mean 39.2% vital bone formation. After nearly

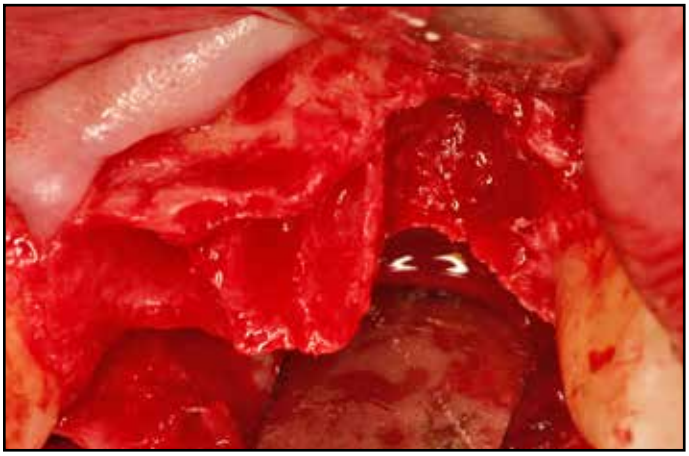


Figure 11a: Combination bony defect in anterior mandible.

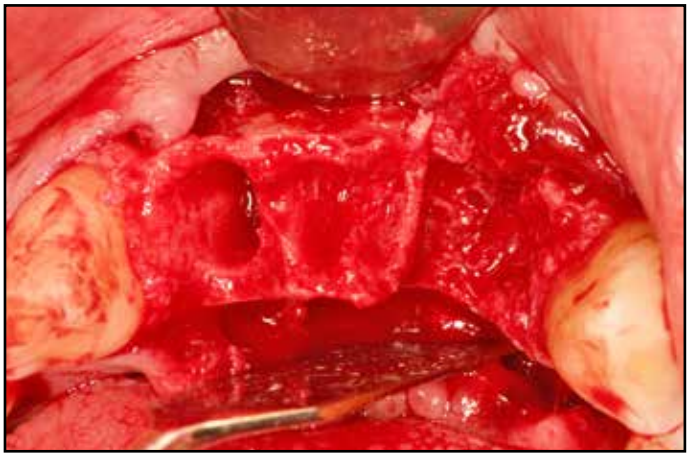


Figure 11b: Combination bony defect in anterior mandible (alternative view).

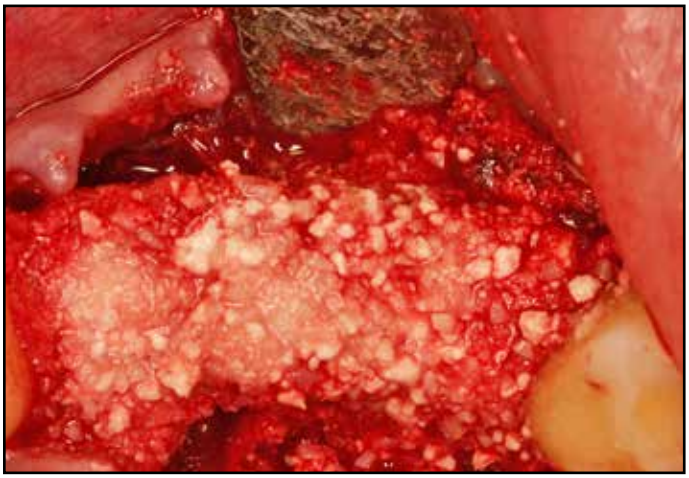


Figure 11c: Combination bony defect in anterior mandible grafted with particulate allograft.

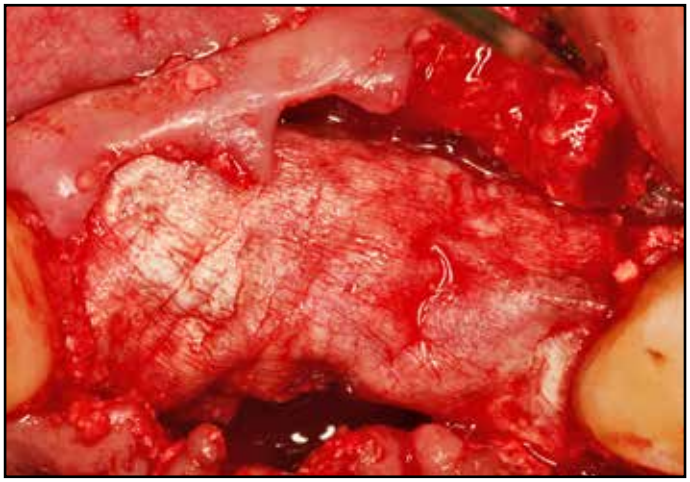


Figure 11d: Grafted combination bony defect in anterior mandible covered with collagen membrane.

3 years following the publication of this paper, dental implants placed into these dHACM grafted sites have a 100% survival rate.

Another site preservation study was released in 2014 comparing BioXclude™ dHACM barrier to dense polytetrafluoroethylene (d-PTFE) barrier.[73] This prospective, intra-patient, clinical evaluation involved 9 patients with 22 sites (11 per group) where implants were placed at 12-14 weeks. Results from this study revealed that BioXclude™ treated sites had on average more bone volume and less resorption of alveolar ridge width compared to sites treated with d-PTFE barriers. Furthermore, sites treated with the resorbable BioXclude™ dHACM did not require follow up procedures for barrier removal

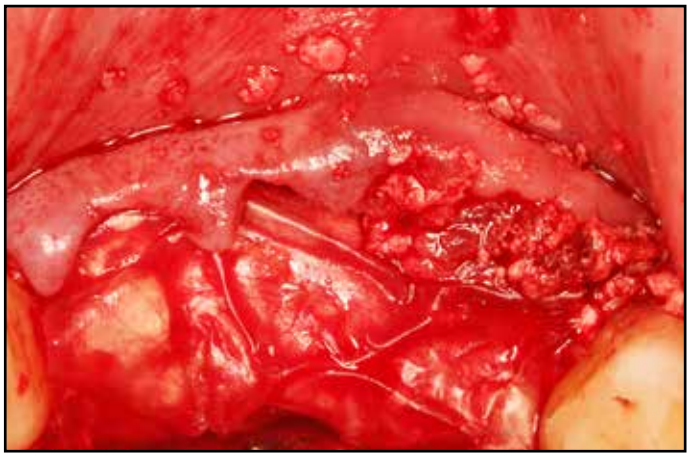


Figure 11e: BioXclude™ dHACM placed on top of collagen membrane to achieve modified guided bone regeneration technique.

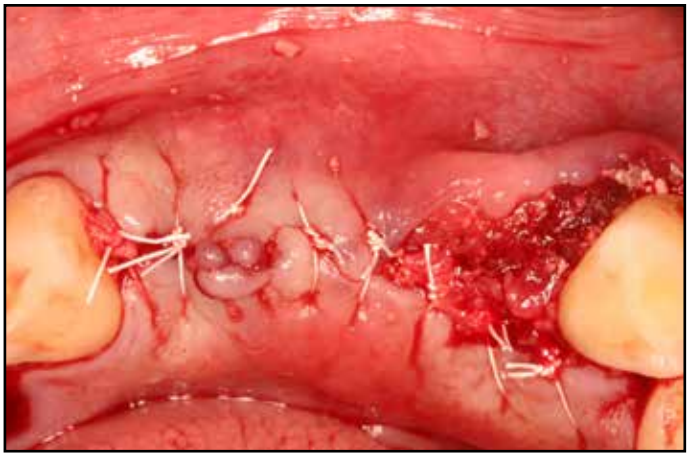


Figure 11f: Closure of modified guided bone regeneration site utilizing collagen membrane overlaid with BioXclude™ dHACM (Note non-primary closure at distal aspect with BioXclude™ dHACM exposed).

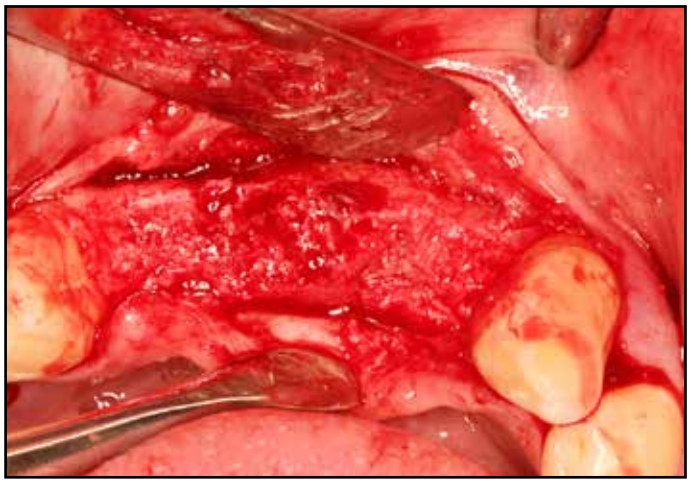


Figure 11g: Bone healing of modified GBR treated combination bony defect in anterior mandible at 3 months healing.

unlike the non-resorbable d-PTFE barriers.

Guided Tissue Regeneration of Periodontal and Peri-Implantitis Defects

The first published utilization of amnion-chorion for guided tissue regeneration (GTR) treatment of a periodontal defect occurred in early 2011.[74,75] In this case report, Holtzclaw utilized BioXclude™ dHACM to treat an intrabony periodontal defect of a mandibular molar that had probing depth measurements of 9mm and 10mm of clinical attachment loss (CAL). Following full thickness mucogingival flap elevation, the intrabony defect was thoroughly degranulated. The cleansed defect was grafted with mineralized freeze dried bone allograft and covered with a single layer of BioXclude™ dHACM. After 6 months of healing, probing depth and clinical attachment improved by 6mm and 5mm respectively. With regular periodontal maintenance therapy, these gains have been maintained for nearly 6 years as of the writing of this paper.

A 2013 study by Holtzclaw[27] involved 114 patients who were treated with GTR therapy from March 2010 to October 2011. Of these patients, 64 were treated with BioXclude™ dHACM combination GTR therapy and had ≥12 months of follow-up. All patients

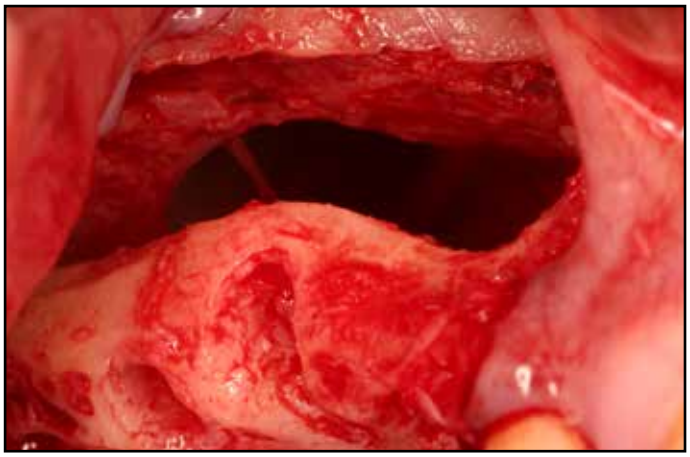


Figure 12a: Perforated Schneiderian membrane during maxillary sinus lift.

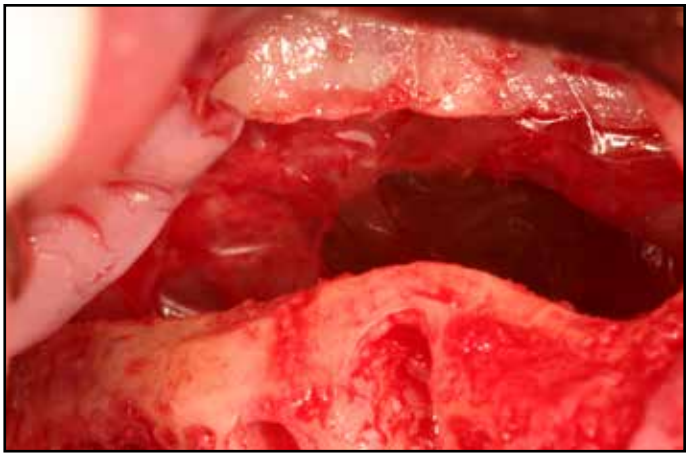


Figure 12b: BioXclude™ dHACM being used to repair perforated Schneiderian membrane.

were diagnosed with localized moderate-to-severe chronic periodontitis and exhibited radiographic evidence of ≥1mm vertical osseous defects. Treatment involved thorough degranulation of intrabony periodontal defects and placement of bone allograft covered by BioXclude™ dHACM (Figures 7a-d). Clinical measurements 12 months after surgery revealed an average probing depth reduction of 5.06 ± 1.37 mm and clinical attachment level improvement of 4.61 ± 1.29 mm.

The treatment of peri-implantitis intrabony defects follows very similar principles to the treatment of periodontal intrabony defects. While some variations do exist in terms of implant surface detoxification versus natural tooth detoxification, the basic concept and treatment tenets remain similar. Figures 8a-g demonstrate how BioXclude™ dHACM is used for treatment of a peri-implantitis intrabony defect.

Guided Bone Regeneration

Guided bone regeneration (GBR) entails augmentation of edentulous sites which are of inadequate dimensions for the placement of dental implants. A vast multitude of techniques exist to accomplish GBR and most call for long lasting barrier materials including various collagens, pericardial tissues, acellular dermal matrices, titanium mesh, resorbable mesh, expanded polytetrafluoroethylene (e-PTFE), and d-PTFE.[76] Traditional tenets of GBR include barriers that create space maintenance, wound stability, epithelial exclusion, and graft containment. When using BioXclude™ dHACM for GBR (Figures 9a-e), an alternative thought process toward these tenets must be considered. In terms of graft containment, BioXclude™ dHACM satisfactorily satisfies the tenet. In terms of wound stability, BioXclude™ dHACM performs exceptionally well as demonstrated by Holtzclaw et al.[77] in a recent modified replication of the classic 1968 flap attachment study. In terms of epithelial exclusion, BioXclude™ dHACM seems to oppose the tenet as its high Laminin and Laminin-5content[30,70] actually encourages epithelial cell proliferation. While this may seem like a detriment, the high Laminin and Laminin-5

content of BioXclude™ dHACM allows epithelial cells to rapidly migrate across its chorioamniotic matrix in such a fashion that the cells do not invade the underlying bone graft. This has been verified in multiple studies that show bone augmentation sites treated with BioXclude™ dHACM have histologic core samples of similar composition to sites that are treated with other traditional techniques.[65,72] Once these epithelial cells come into contact with one another, contact inhibition halts their migration,[78] effectively sealing the underlying bone graft material and further preventing the migration of epithelial cells into the bone graft. In this sense, BioXclude™ dHACM satisfies the tenet of epithelial cell exclusion from the underlying bone graft in spite of the fact that it actually promotes the proliferation of epithelial cells. Finally, in terms of space maintenance, BioXclude™ dHACM occasionally satisfies this tenet, but not always, due to its thin 300µm cross sectional thickness and pliability. With small 3 walled bony defects, BioXclude™ dHACM can be effectively used as the existing bony walls provide space maintenance for GBR (Figures 10a,b). With larger 3 walled defects and 1 or 2 walled defects, a modified GBR technique may be employed whereby a stiffer barrier such as collagen or titanium is utilized for space maintenance and BioXclude™ dHACM is overlaid on top of the barrier (Figures 11a-g). Overlaying the stiffer barrier with BioXclude™ allows the modified GBR technique to achieve space maintenance while still retaining the multitude of wound healing benefits provided by dHACM. The modified GBR technique was demonstrated by Holtzclaw[79] in a 2016 publication that utilized a combination of titanium mesh overlaid with BioXclude™ dHACM for the treatment of a severe alveolar ridge defect with recombinant human bone morphogenetic protein (rh-BMP2).

Maxillary Sinus Augmentation and Repair

Pneumatization of the maxillary sinus is a common complication of edentulism in the posterior maxilla. To facilitate placement of dental implants in the pneumatized maxillary sinus, augmentation is often required. The process of maxillary sinus augmentation requires careful elevation of the Schneiderian membrane. With rates ranging from 11% to 56%, perforation of the Schneiderian membrane is the most common complication associated with maxillary sinus augmentation procedures[80] and has been linked to a variety of problems including the need for procedure abortion, decreased bone formation, and a possible reduction in dental implant survival.[81,82] BioXclude™ dHACM is uniquely qualified for repair of Schneiderian membrane perforations. In addition to its aforementioned wound healing properties which can augment the sinus grafting process, BioXclude™ dHACM is of similar thickness to the Schneiderian membrane and its self-adherent nature allows the barrier to readily attach to sinus membrane sans suture. Utilization of BioXclude™ dHACM for the repair of Schneiderian membrane perforations (Figures 12a,b) was first demonstrated by Holtzclaw[69] in a 2014. In this controlled split mouth case report, healing outcomes were evaluated for bilateral lateral window sinus augmentations performed in a single patient. One sinus was augmented with the Schneiderian membrane intact while the contralateral sinus was augmented with a perforated Schneiderian membrane that was

repaired with BioXclude™ dHACM. The non-perforated sinus augmentation healed with more bone height and a denser, more uniform fill compared to the augmentation repaired with BioXclude™ dHACM. Both sinuses, however, had adequate healing to permit placement of multiple dental implants to support an immediately loaded restoration. It must be noted that with traditional treatment, the large perforation of the Schneiderian membrane in this Case Report would have resulted in abortion of the sinus augmentation. Utilization of BioXclude™ dHACM allowed the procedure to be completed on the same day and produced results that allowed for success implant delivery. After nearly four years of function, implants in both sinuses have demonstrated zero complications and the prosthesis continues to function well.

A second much larger publication examining the utilization of BioXclude™ dHACM for the repair of perforated Schneiderian membranes was published by Holtzclaw[68] in 2015. In this publication, a consecutive retrospective record review was performed of all maxillary sinus augmentations performed during a 5 year period. Eighty three cases were identified with a total of 104 sinus augmentations, of which nine perforations were noted. None of the nine cases were aborted mid-procedure and all perforations were repaired with BioXclude™ dHACM. All cases were augmented with a combination of allograft and xenograft particulate bone. A total of 23 dental implants were placed in the augmented sinuses with perforated Schneiderian membranes and a one failure was noted according to Albrektsson success criteria. A total of 158 dental implants were placed in non-perforated augmented sinuses with a total of three failures noted.

Treatment of Gingival Recession

Treatment of gingival recession via non-autogenous methods has long been sought as a means of reducing second site surgical morbidity for patients. A number of products have been used in attempts to achieve this goal with varying degrees long term results.[83-85] Many studies have evaluated the use of amnion[86-89] for the treatment of gingival recession including studies that examined the use of first generation BioXclude™ precursor dehydrated human amnion membrane (dHAM). In 2009, Gurinsky[67] performed a 5 patient case series in which dHAM was used for root coverage procedures in lieu of traditional autogenous connective tissue grafts. The average gingival defect size treated was 3.3mm (± 0.84). At three month there was an average increase of 3.2 mm (±1.71) of new gingival tissue representing 97% (± 0.5) root coverage. More recently in 2016, Pundir et al.[90] performed a split-mouth case series in which mucogingival defects were treated with either amnion or chorion allografts used in conjunction with coronally advanced flaps. After 6 months of healing, 9 of the 12 treated defects showed 100% root coverage with no statistically significant difference between the two groups. While these studies and others have evaluated both amnion and chorion allografts separately for root coverage, no know studies have evaluated laminated dHACM for this purpose.

Future Possibilities in Dental Treatment

While dHACM has now proven efficacious for a variety of dental procedures, the unique

properties of this product are still being evaluated in a number of other dental applications. Currently, research with dHACM is being conducted for treatment of the following: oroantral communications, temporomandibular disorders, nerve injury, non-healing soft tissue defects, mucogingival root coverage, increasing zone of keratinized gingiva, and non-surgical treatment of periodontal disease.

CONCLUSIONS

Just as medicine continues to expand its utilization of chorio-amniotic products, dentistry is doing the same. In their own right, the extremely high growth factor content, antibacterial properties, angiogenic properties, anti-inflammatory properties, and pain reduction properties make dHACM an extremely effective product. For dentistry specifically, when these properties are combined with the fact that dHACM BioXclude™ can be left exposed to the oral cavity, can touch root surfaces, is self-adherent, and bioabsorbable, the product truly offers a number of unique and useful benefits.

Correspondence:
Dr. Dan Holtzclaw
dholtzclaw@atximplants.com

Disclosure
D Holtzclaw reports a financial interest in Snoasis Medical. R Tofe reports a financial interest in Snoasis Medical.

References

1. Davis J. Skin transplantation with a review of 550 cases at the Johns Hopkins Hospital. Johns Hopkins Hospital Report. 1910;15:307–310.
2. Sorsby A, Symons HM. Amniotic membrane grafts in caustic burns of the eye (burns of the second degree). Br J Ophthalmol. 1946;30:337–345.
3. Sorsby A, Haythorne J, Reed H. Further experience with amniontic membrane grafts in caustic burns of the eye. Br J Ophthalmol 1947;31(7):409-18.
4. Liu FS, CH'EN YC, LIU JH. The use of dehydrated amniotic graft after radical mastoidectomy. Chin Med J 1959;78(5):435-8.
5. Catalano GB, Conticello S. The long-term results of myringoplasty with amnion graft. Otorinolaringologie 1969;14(2):97-102.
6. Zelen CM, Serena TE, Denoziere G, Fetterolf DE. A prospective randomized comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers. Int Wound J 2013;10:502–7.
7. Zelen CM. An evaluation of dehydrated human amniotic membrane allografts in patients with DFUs. J Wound Care 2013;22 347–8,350–1
8. Wisco OJ. Case series: The use of a dehydrated human amnion/chorion membrane allograft to enhance healing in the repair of lower eyelid defects after Mohs micrographic surgery. JAAD Case Rep. 2016 Jul 27;2(4):294-7.
9. Miranda EP, Friedman A. Dehydrated Human Amnion/Chorion Grafts May Accelerate the Healing of Ulcers on Free Flaps in Patients With Venous Insufficiency and/or Lymphedema. Eplasty. 2016 Sep 7;16:e26.
10. Nisolle M, Donnez J. Vaginoplasty using amniotic membranes in cases of vaginal agenesis or after vaginectomy. J Gynecol Surg 1992;8(1):25-30.
11. Hensle TW, Seaman EK.Vaginal reconstruction in children and adults. Tech Urol 1995;1(4):174-80.
12. Patel VR, Samavedi S, Bates AS, Kumar A, Coelho R, Rocco B, Palmer K. Dehydrated Human Amnion/Chorion Membrane Allograft Nerve Wrap Around the Prostatic Neurovascular Bundle Accelerates Early Return to Continence and Potency Following Robot-assisted Radical Prostatectomy: Propensity Score-matched Analysis. Eur Urol 2015;67(6):977-80.
13. Dua H, Gomes J, King A, et al. The amniotic membrane in opthamology. Surv Ophthalmol 2004;49(1):51-77.
14. Niknejad H, Peirovi H, Jorjani M, et al. Properties of the amniotic membrane for potential use in tissue engineering. Eur Cell Mater 2008;15:88-99.
15. Hodde J. Naturally occurring scaffolds for soft tissue repair and regeneration. Tiss Eng 2002; 8(2): 295-308.
16. Parry S, Strauss J. Premature rupture of the fetal membranes. N Engl J Med 1998;338(10):663-70.
17. Chua W, Oyen M. Do we know the strength of the chorioamnion? A critical review and analysis. Eur J Obstet Gynecol Repod Biol 2009;144:128-133.
18. Fournel S, Aguerre-Girr M, Huc X et al. Cutting edge: Soluble HLA-G1 triggers CD95/CD95 ligand-mediated apoptosis in activated CD8+ cells by interacting with CD8. J Immunol 2000; 164:6100–6104.
19. Makrigiannakis A, Zoumakis E, Kalantaridou S et al. Corticotropin-releasing hormone promotes blastocyst implantation and early maternal tolerance. Nat Immunol 2001; 2:1018–1024.
20. Thellin O, Coumans B, Zorzi W et al. Tolerance to the foeto-placental 'graft': Ten ways to support a child for nine months. Curr Opin Immunol 2000; 12:731–737.
21. Kubo M, Sonoda Y, Muramatsu R, Usui M. Immunogenicity of Human Amniotic Membrane in Experimental Xenotransplantation. IOVS 2001; 42(7): 1539-1546.
22. Park CY, Kohanim S, Zhu L, Gehlbach PL, Chuck RS. Immunosuppressive Property of Dried Human Amniotic Membrane. Ophthalmic Res 2009;41: 112–113.
23. U.S. Food and Drug Administration, Department of Health and Human Services. Part 1271 Human Cells, Tissue, and Cellular and Tissue-Based Products. Code of Federal Regulations 2007; Title 21(r81):712-738.
24. U.S. Food and Drug Administration, Department of Health and Human Services. Subpart D: current good tissue practice–human cells, tissue, and cellular and tissue-based product establishments. Fed Regist 2004;69(226):68612-68688.
25. U.S. Food and Drug Administration, Department of Health and Human Services. Subpart C: donor eligibility, part 1271–human cell, tissue, and cellular and tissue based products. Fed Regist 2004;69(101):29786-29834.
26. A Primer on Amniontic Membrane Regenerative Healing. Koob T, et al. MiMedx/Color House Graphics; Grand Rapids, Michigan 2015.
27. Holtzclaw D, Toscano N. Amnion–Chorion Allograft Barrier Used for Guided Tissue Regeneration Treatment of Periodontal Intrabony Defects: A Retrospective Observational Report. Clin Adv Periodont 2013;3(3):131-137.
29. Chen E, Tofe A. A Literature Review on the Safety and Biocompatibility of Amnion Tissue. J Implant Adv Clin Dent 2010;2(3):67-75.
30. MiMedx Research Report. MM-RD-00021: Impacts of Terminal Sterilization on dHACM.
31. MiMedx Research Report. MM-RD-00022: EpiFix and AmnioFix: Total Growth Factor Content.
32. Solomon A, Rosenblatt M, Monroy D, et al. Suppression of interleukin 1á and interleukin 1â in human limbal epithelial cells cultured on the amniotic membrane stromal matrix. Br J Ophthalmol 2001; 85: 444-449.
33. Park WC, Tseng SCG. Modulation of acute inflammation and keratocyte death by suturing, blood, and amniotic membrane in PRK. Invest Ophthalmo Vis Sci 2000; 41(10): 2906-2914.
34. Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits proteinase activity on wound healing following acute corneal alkali burn. Exp Eye Res 2000;70(3):329-37.
35. Tseng SC. Amniotic membrane transplantation for ocular surface reconstruction. Biosci Rep 2001; 21(4):481-9.
36. Güell JL, Morral M, Gris O, Elies D, Manero F. Treatment of symptomatic bullous keratopathy with poor visual prognosis using a modified Gundersen conjunctival flap and amniotic membrane. Ophthalmic Surg Lasers Imaging 2012;43(6):508-12.
37. Koob TJ, Rennert R, Zabek N, Massee M, Lim JJ, Temenoff JS, Li WW, Gurtner G. Biological properties of dehydrated human amnion/chorion composite graft: implications for chronic wound healing. Int Wound J 2013;10(5):493-500.
38. Koob TJ, Lim JJ, Massee M, Zabek N, Denozière G. Properties of dehydrated human amnion/chorion composite grafts: Implications for wound repair and soft tissue regeneration. J Biomed Mater Res B Appl Biomater 2014;102(6):1353-62.
39. Tehrani FA, Ahmadiani A, Niknejad H.The effects of preservation procedures on antibacterial property of amniotic membrane. Cryobiology 2013;67(3):293-8.
40. Stock SJ, Kelly RW, Riley SC, Calder AA. Natural antimicrobial production by the amnion. Am J Obstet Gynecol 2007;196(3):255.e1-6.
41. Kjaergaard N, Hein M, Hyttel L, Helmig RB, Schønheyder HC, Uldbjerg N, Madsen H. Antibacterial properties of human amnion and chorion in vitro. Eur J Obstet Gynecol Reprod Biol 2001;94(2):224-9.
42. Mermet I, Pottier N, Sainthillier JM, Malugani C, Cairey-Remonnay S, Maddens S, Riethmuller D, Tiberghien P, Humbert P, Aubin F. Use of amniotic membrane transplantation in the treatment of venous leg ulcers. Wound Repair Regen 2007;15(4):459-64.
43. Sikkerimath BC, Dandagi S, Gudi SS, Jayapalan D. Comparison of vestibular sulcus depth in vestibuloplasty using standard Clark's technique with and without amnion as graft material. Ann Maxillofac Surg 2012;2(1):30-5.
44. Eskandarlou M, Azimi M, Rabiee S, Seif Rabiee MA. The Healing Effect of Amniotic Membrane in Burn Patients. World J Plast Surg 2016;5(1):39-44.
45. Velez I, Parker WB, Siegel MA, Hernandez M. Cryopreserved amniotic membrane for modulation of periodontal soft tissue healing: a pilot study. J Periodontol 2010;81(12):1797-804.
46. Burgos H. Angiogenic and growth factors in human amnio-chorion and placenta. Eur J Clin Invest 1983;13(4):289-96.
47. Burgos H. Angiogenic factor from human term placenta. Purification and partial characterization. Eur J Clin Invest 1986;16(6):486-93.
48. Yamahara K, Harada K, Ohshima M, Ishikane S, Ohnishi S, Tsuda H, Otani K, Taguchi A, Soma T, Ogawa H, Katsuragi S, Yoshimatsu J, Harada-Shiba M, Kangawa K, Ikeda T. Comparison of angiogenic, cytoprotective, and immunosuppressive properties of human amnion- and chorion-derived mesenchymal stem cells. PLoS One 2014; 14;9(2):e88319.
49. Cheung CY, Brace RA. Developmental expression of vascular endothelial growth factor and its receptors in ovine placenta and fetal membranes. J Soc Gynecol Investig 1999;6(4):179-85.

50. Ahmed A, Li XF, Dunk C, Whittle MJ, Rushton DI, Rollason T. Colocalisation of vascular endothelial growth factor and its Flt-1 receptor in human placenta. Growth Factors 1995;12(3):235-43.
51. Marvin KW, Keelan JA, Eykholt RL, Sato TA, Mitchell MD. Expression of angiogenic and neurotrophic factors in the human amnion and choriodecidua. Am J Obstet Gynecol 2002;187(3):728-34.
52. Koob TJ, Lim JJ, Massee M, Zabek N, Rennert R, Gurtner G, Li WW. Angiogenic properties of dehydrated human amnion/chorion allografts: therapeutic potential for soft tissue repair and regeneration. Vasc Cell 2014;1;6:10.
53. Alexiadou K, Doupis J. Management of Diabetic Foot Ulcers. Diabetes Ther 2012;3(1):4.
54. DiDomenico LA, Orgill DP, Galiano RD, Serena TE, Carter MJ, Kaufman JP, Young NJ, Zelen CM. Aseptically Processed Placental Membrane Improves Healing of Diabetic Foot Ulcerations: Prospective, Randomized Clinical Trial. Plast Reconstr Surg Glob Open 2016;4(10):e1095.
55. Moxey PW, Gogalniceanu P, Hinchliffe RJ, et al. Lower extremity amputations–a review of global variability in incidence. Diabet Med 2011;28:1144–1153.
56. Zelen CM, Serena TE, Snyder RJ. A prospective, randomised comparative study of weekly versus biweekly application of dehydrated human amnion/chorion membrane allograft in the management of diabetic foot ulcers. Int Wound J 2014;11(2):122-8.
57. 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(5):737-44.
58. Zelen CM, Gould L, Serena TE, Carter MJ, Keller J, Li WW. A prospective, randomised, controlled, multi-centre comparative effectiveness study of healing using dehydrated human amnion/chorion membrane allograft, bioengineered skin substitute or standard of care for treatment of chronic lower extremity diabetic ulcers. Int Wound J 2015;12(6):724-32.
59. Zelen CM, Serena TE, Gould L, Le L, Carter MJ, Keller J, Li WW. Treatment of chronic diabetic lower extremity ulcers with advanced therapies: a prospective, randomized, controlled, multi-centre comparative study examining clinical efficacy and cost. Int Wound J 2016;13(2):272-82.
60. DiDomenico LA, Orgill DP, Galiano RD, Serena TE, Carter MJ, Kaufman JP, Young NJ, Zelen CM. Aseptically Processed Placental Membrane Improves Healing of Diabetic Foot Ulcerations: Prospective, Randomized Clinical Trial. Plast Reconstr Surg Glob Open 2016;4(10):1095.
61. Penny H, Rifkah M, Weaver A, Zaki P, Young A, Meloy G, Flores R. Dehydrated human amnion/chorion tissue in difficult-to-heal DFUs: a case series. J Wound Care 2015;24(3):104-111.
62. Selvig KA, Kersten BG, Chamberlain AD, Wikesjö UM, Nilvéus RE.Regenerative surgery of intrabony periodontal defects using ePTFE barrier membranes: scanning electron microscopic evaluation of retrieved membranes versus clinical healing. J Periodontol 1992;63(12):974-8.
63. Simion M, Trisi P, Maglione M, Piattelli A. A preliminary report on a method for studying the permeability of expanded polytetrafluoroethylene membrane to bacteria in vitro: a scanning electron microscopic and histological study. J Periodontol 1994;65(8):755-61.
64. Sela MN, Kohavi D, Krausz E, Steinberg D, Rosen G. Enzymatic degradation of collagen-guided tissue regeneration membranes by periodontal bacteria. Clin Oral Implants Res 2003;14(3):263-8.
65. Holtzclaw D, Toscano N. BioXclude Placental Allograft Tissue Membrane Used in Combination with Bone Allograft for Site Preservation: A Case Series. J Implant Adv Clin Dent 2011;3(3):35-50.
66. Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J. Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. Clin Oral Implants Res 2005;16:369-378.
67. Gurinsky B. A novel dehydrated amnion allograft for use in the treatment of gingival recession: An observational case series. J Implant Adv Clin Dent 2009;1:11-6.
68. Holtzclaw D. Maxillary sinus membrane repair with amnion-chorion barriers: A retrospective case series. J Perio 2015; 86(8): 936-940.
69. Holtzclaw D. Open sinus lift healing comparison between a non-perforated Schneiderian membrane and a perforated Schneiderian membrane repaired with amnion-chorion allograft barrier: A controlled, split mouth case report. J Implant Adv Clin Dent 2014;6(8):11-21.
70. Xenoudi P and Lucas M. IADR Meeting, Abstract #146797. San Diego, CA. March 16-19 2011.
71. Holtzclaw D, Toscano N. BioXclude Placental Allograft Tissue Membrane Used in Combination with Bone Allograft for Site Preservation: A Case Series. J Implant Adv Clin Dent 2011;3(3):35-50.
72. Wallace S, Cobb C. Histologic and Computed Tomography Analysis of Amnion-Chorion Membrane in Guided Bone Regeneration Socket Augmentation. J Implant Adv Clin Dent 2011;3(6):61-72.
73. Hassan, M, Blanchard S, Prakasam, S. AAP Poster Presentation. Sept 19-22, 2014, San Francisco, CA. University of Indiana, Department of Periodontics.
74. Holtzclaw D. Use of Amnion-Chorion in Guided Tissue Regeneration. Poster Presentation. Post #1102334 American Academy of Periodontology 97th Annual Meeting 2011, Miami Beach, FL.
75. Holtzclaw D. BioXclude™ Placental Allograft Tissue Membrane Used in Combination with Bone Allograft for Guided Tissue Regeneration Treatment of Periodontal Intrabony Defect: A Case Report. White Paper 2011; Snoasis Medical, Denver, Colorado.
76. Soldatos NK, Stylianou P, Koidou VP, Angelov N, Yukna R, Romanos GE. Limitations and options using resorbable versus nonresorbable membranes for successful guided bone regeneration. Quintessence Int. 2016 Nov 10. Epub ahead of print.
77. Holtzclaw D, Hinze F, Toscano N. Gingival flap attachment healing with amnion-chorion allograft membrane: A controlled, split mouth case report replication of the classic 1968 Hiatt study. J Imp Adv Clin Dent 2012; 4(5):19-25.
78. Puliafito A, Hufnagel L, Neveu P, Streichan S, Sigal A, Fygenson DK, Shraiman BI. Collective and single cell behavior in epithelial contact inhibition. Proc Natl Acad Sci U S A 2012;17;109(3):739-44.
79. Holtzclaw D. Dehydrated Human Amnion-Chorion Barrier used to Assist Mucogingival Coverage of Titanium Mesh and rhBMP-2 Augmentation of Severe Maxillary Alveolar Ridge Defect: A Case Report. J Implant Adv Clin Dent 2016;8(4):6-16.
80. Toscano N, Holtzclaw D, Rosen P. The effect of piezoelectric use on open sinus lift perforation: A retrospective evaluation of 56 consecutively treated cases from private practices. J Periodontol 2010; 81(1):167-171.
81. Proussaefs P, Lozada J, Kim J, Rohrer M. Repair of perforated sinus membrane with resorbable collagen membrane: A human study. Int J Oral Maxillofac Implants 2004; 19(3):413-420.
82. Hernandez-Alfaro F, Torradeflot MM, Marti C. Prevalence and management of Schneiderian membrane perforations during sinus lift procedures. Clin Oral Implants Res 2008; 19:91-98.
83. Harris RJ. A comparative study of root coverage obtained with an acellular dermal matrix versus a connective tissue graft: results of 107 recession defects in 50 consecutively treated patients. Int J Periodontics Restorative Dent 2000;20(1):51-9.
84. Allen EP. Subpapillary continuous sling suturing method for soft tissue grafting with the tunneling technique. Int J Periodontics Restorative Dent 2010;30(5):479-85.
85. Aroca S, Keglevich T, Barbieri B, Gera I, Etienne D. Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. J Periodontol 2009;80(2):244-52.
86. Rucha S, Sowmya NK, Mehta DS. Amnion membrane for coverage of gingival recession: A novel application. Contemp Clin Dent 2014;5:293-5.
87. Chopra A, Thomas BS. Amniotic membrane: A novel material for regeneration and repair. J Biomim Biomater Tissue Eng 2013;18:1-8.
88. Borghe A, Gardella JP. Thick gingival autograft for the coverage of gingival recession: A clinical evaluation. Int J Periodontics Restorative Dent 1990;10:216-29.
89. Ghahroudi AA, Khorsand A, Rokn AR, Sabounchi SS, Shayesteh YS, Soolari A. Comparison of amnion allograft with connective tissue graft for root coverage procedures: A double-blind, randomized, controlled clinical trial. J Int Acad Periodontol 2013;15:101-12.
90. Aena Jain Pundir, Vandita Agrawal, Siddharth Pundir, Vikas Diwan, and Sonika Bodhi. Comparative Evaluation of the Efficacy of Human Chorion and Amnion With Coronally Advanced Flap for Recession Coverage: A Case Series. Clin Adv Periodont 2016;6(3):118-126.