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The Scope of Interactive Orthodontics
Chapter 12
In vivo Tissue Engineering for Orthodontists II: ©

A "Stem Cell" Protocol, Theory, and Rationale

by

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This paper is dedicated to the noble and selfless inspiration of our friend and mentor
Dr. Donald Enlow, Professor Emeritus, Case Western Reserve University
and the abiding scholastic legacy which he left and we are privileged to sustain.

"What nobler employment, or more valuable to the state,
than that of the man who instructs the rising generation?
-- Marcus Tillius Cicero (106-43 BC)

In vivo tissue engineering for orthodontists II: ©

"Stem Cell" Therapy, Theory, Rationale and Clinical Protocol

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Introduction:

Interdisciplinary study of orthodontics and periodontics asks two basic questions: (1) what are the effects of orthodontic tooth movement (OTM) on periodontal health? (2) How does tooth movement affect alveolar form with changes in root position and periodontal grafting? A third question, "How does this interscholastic discipline eliminate outdated dogma and parochial bias?" is subsumed in the first two. The effects of OTM on the alveolus bone at the gross anatomical level has been well documented in the periodontal literature since the 1970's (Brown, 1973; Ingber, 1974; Ingber, 1976) and nicely summarized by Mihram in the 1990's (Mihram, 1997). More recently, in numerous global venues, both clinical (Wilcko, 2001; Ducker, 1975; Merrill, 1976; Generson, 1978; Mostaza, 1985; Anholm,1986; Yoshikawa, 1987; Matsuda, 1989;Liou, 1998; Owen, 2001;Fulk, 2002; Hajji, 2002; Kasewicz, 2004; Iseri, 2005; Ahlawat, 2006, Dosanjh, 2006) and experimental studies, (Bell, 1972; Nakanishi, 1982; Gantes, 1990; Kawakami, 1996; Twaddle, 2001; Machado, 2002; Machado, 2002; Navarov, 2004; Kelson, 2005; Sebaoun, 2006; Ferguson, 2006;Oliveira, 2006) document the efficacy of selective alveolar decortication (SAD) with and without bone grafting to accelerate orthodontic tooth movement and increase alveolar mass. The surgical facilitation of OTM was first described by Cunningham in 1894, and iterations appeared in the scientific literature thereafter, mainly in German (Cohn-Stock, 1921; Bichlmayr, 1931; Ascher, 1947; Neuman, 1955). An influential clinical description of this successful surgical manipulation was published in the American literature, nearly 50 years ago. (Kole, 1959) and lay dormant until analyzed at Loma Linda University in the 1980's.

This recently popularized surgical approach to dentofacial orthopedics is especially popular among younger orthodontists and residents, as each generation seems to make claims on the equity of new

science understandable. The aim of this chapter is to synthesize these data into the context of the emerging sciences of tissue engineering and stem cell therapy.

Traditional Concepts in Orthodontic-Periodontal Interactions

Although the data have grown in volume very rapidly, the meritoriously slow pace of curricula change in dental education has lent a necessary social and professional stability. There is an old adage in the U.S. Army that says, "It is never wise to be first or last in line'. The reader should be reassured to know that the content herein lies at neither polar position. It is not first because it builds on medical orthopedic literature and basic biology from PhD-level scholars. Yet it certainly does not represent the orthodontics of our fathers. Thus, it is presented as a naturally evolved body of clinical protocols and biologic standards which collectively represent sufficient *gravitas* to justify immediate implementation into practice. The emergence of the clinical protocols are constantly undergoing refinement and but continue to rest on the firmament of logic and remain close to the edge of a growing body of cellular and molecular biologic innovation.

It would be disadvantageous to launch into emerging standards of excellence without a review of traditional concepts of orthodontic and periodontal interactions. Then we may move the story further forward by discussing both tissue engineering and stem cell therapy (SCT) as the areas which unite two specialties into surgical dentofacial orthopedics. The traditional pedagogical rubric "ortho-perio interactions" is still seen as a curiosity to reactionary artisans. But to progressives, it is an old prototype of clinical orthodontics. Still, a review provides a bridge between the wire-bending art of yesterday with the new horizon of tissue engineering (TE) and the rising sun of stem cell therapy for orthodontists.

Bone and Attachment Level in Health and Disease

The periodontium consists of the gingival unit and, apical to that, the periodontal attachment apparatus. (Fig. 1) The gingival unit generally moves with the tooth and contains, when not bleeding on probing, a sulcus of 1-3 mm in health. When everted (prolapsed) the sulcus tissue appears as the proverbial "Red Patch of Atherton" (Fig. 2). This red patch eventually forms a new sulcus and marginal gingiva, moving coronally in health but it can also be a source of attachment loss if bacterial toxins interfere with fibroplasia and crestal osteogenesis. This attachment loss is viewed as "bone loss" in radiographs when about 40% of the alveolar crest is decalcified. After decalcification, the organic matrix is lost, usually permanently. The latter event is conveniently called "bone loss", but more correctly, "attachment loss". Sometimes a long junctional epithelial attachment is observed when bone loss has occurred but tissue tonus is firm enough, like a tight collar, to hold onto the root tightly. (Figure 3) This can be misleading because diagnostic probing of the "bottom" of the sulcus is never achieved with diagnostic probing force of 20-25 grams. As a practical guide, the Michigan-O probe weights about 17 $\pm$ 3 grams, so excessive force to negotiate a pocket is rarely necessary. * The "long junctional epithelial attachment" is thought to be less resistant to bacterial breakdown because the root-epithelial interface, a mucopolysaccharide and hemidesmosomal attachment, is a less formidable defense to bacterial toxins. For the sake of cosmetic appearances in the anterior dentition, some periodontists accept this as a satisfactory, albeit compromised, anatomy. (Fig. 9)

In health, the crest of the alveolar bone is usually about 1 mm apical to the most apical cell of the junctional epithelium (epithelial attachment). This dimension, the sulcus, and a millimeter of healthy junctional epithelium constitute the so-called "biologic width, an inviolable 2-3mm biologic measure of anatomic homeostasis. This dimension must never be violated with bands. If it is encroached upon

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by injudicious band or bracket placement, a destructive cascade of tissue loss ensues. This can initiate periodontal pocket formation, irreversible bone loss and, at worst, a self perpetuating periodontitis. Generally, this "gingival unit" coronal to the bone crest follows movement of the teeth when the periodontium is healthy. In disease however, the tooth moves independent of the the gingival unit and the crestal bone. Because of this relationship, orthodontic therapy can inadvertently move a tooth out of its infected socket by overcoming the adaptive potential of a weakened attachment apparatus. This accelerates attachment loss if periodontal infection is not treated before and during orthodontic therapy (Sanders, 1999).

That is why periodontal health is essential during orthodontic therapy and also explains why recession is often evident as inflammation resolves after debonding. The hypertrophy of tissue edema during therapy often hides the attachment loss and latent recession. The orthodontist's index of suspicion should always make the entire staff vigilant to occult attachment loss and supportive of collaborative therapy by a periodontist or referring dental professional.

Bone Morphing with Orthodontic Tooth Movement

The orthodontist usually sees fixed appliances simply as ortho-*dontic* apparati for moving tooth crowns for cosmetic advantage. But, increasingly, they are seen as ortho-*pedic* devices, restructuring the plastic alveolus bone to more physiologic form. The tendency of alveolar bone to move with the tooth and its engineering potential was noted on casts by Hom (1983), validated *in vivo* by Kokich (2005) and discussed as phenotype change by Williams (2008). Interestingly, the conceptual basis was widely discussed by Moss in the 1960's and revisited in terms of an epigenetic mechanism (Moss, 1997d). The phenomenon is employed for so-called "crown lengthening", i.e. the exposure of more anatomical crown and/or root to facilitate restorative care. This therapy makes use of the independent movement of roots out of the bone as mentioned above. Crown lengthening is achieved

in a healthy periodontal attachment apparatus if trans-septal, gingival and superficial crestal periodontal fibers are periodically severed during tooth extrusion. However, this can be a conceptually challenging protocol and deserves some focused study.

Note that when a molar is up-righted to evert a mesial periodontal pocket, an iatrogenic distal pocket can be created if the molar is not extruded symmetrically and the coronal surface reduced in the process. (Fig. 4) When Ingber (1974) first published the idea of force eruption, little was known about how to manage the mesial and distal bone level when a pocket appeared on one interproximal side and the other was healthy. That is, how can one unilaterally (e.g. distally) extrude a root orthodontically out of bone while maintaining the level of attachment on the opposite proximal surface (mesial)? This was explained in subsequent articles by Mihram and Murphy, (2008)

This relationship of the crestal alveolar bone to the root is a critical concept to fathom because it allows correct intuitive judgments by the orthodontist about periodontal health while challenged by a full day of patient demands and biomechanical problems. Psychosocial compliance issues unique to orthodontic offices, especially in an adolescent-based practice, are often more daunting in this regard than the pastoral environment of a surgeons office. Thus, maintaining a quick wit, borne of serious study, is paramount. In the case of “asymmetrical bone loss”, a pocket on one proximal side and healthy sulci on the other, only an “asymmetrical forced eruption” can produce a symmetrically “lengthened” crown, illustrated by successful molar uprighting. (Figs. 5-7)

Asymmetrical forced eruption is achieved by periodically severing the attachment on the healthy side of the root, say the distal, while merely scaling and root planing the tooth surface on the side of the infrabony defect, (in our example, the mesial in Figure 8.) Asymmetrical forced eruption, therapeutically everts the periodontal pocket on the compromised (mesial)surface of the tooth with

periodic fiberotomies preventing both coronal movement of the bone and the creation of an artifactual infrabony defect on the other proximal (distal) side e.g. in Figure 8

The Infected Orthodontic Patient

The reader is directed to an informative survey on this subject published in 2008 by Professors Palomo, Palomo and Bissada. This section relies heavily on that article and reviews its main points. What the authors' argued was that the human mouth is impossible to sterilize, so in the spirit of "universal precaution", all patients are considered always "infected", more or less. Since the authors published their survey an appliance-friendly floss holder* and a number of pharmacologic agents have become more popular. But medical advances in the study of chronic inflammation and global cultural sophistication have made periodontal management of appliance-induce infection a much more important intellectual issue than simply instruction in flossing. So the most pressing issue for the practicing orthodontist is this: Given the ubiquitous and constant field of infected tissue in which the one operates, how can one minimize irreversible tissue damage?

Once informed and coached in oral hygiene, the proximate cause of most dental disease and bone loss is a matter of poor patient commitment, negligently ignoring the doctors' advice. Although pharmacologic agents, used *in situ*, are legitimate, their effect is fleeting and these expensive supplements may be impractical for many. On the other hand, in the long run they represent an excellent investment because they are effective in preventing or mitigating sudden exacerbations of latent disease. If using pharmacologic media, remember that the ultimate goal of any antibacterial therapy is to make a niche for commensal organisms which will "crowd out" more virulent pathogens. Caveat: long term reliance on pharmaceuticals risks the development of bacterial strains resistant to any pharmaceutical. Because of this limitation, most periodontists prefer that patients use

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mechanical methods of dental plaque (bacterial biofilm) removal, daily and assiduously.

Benign commensal bacteria are relatively welcomed residents when they limit their reversible damage to the gingival unit. But virulent pathogenic forms and commensal organisms cannot be well distinguished in oral bacterial biofilms Therefore universal precaution, it is argued, similar to those used in other infection-prone environments, should be employed with all orthodontic patients. The key to success is to keep bleeding on probing (BOP) to a minimum. Since the task of infection control rests on undependable patient compliance, every patient should be informed of the risks of periodontal damage and explicitly encouraged to participate in "infection control" by a periodontist, dentist, dental hygienist *and* other trained para-professionals during fixed appliance therapy. Prior to bracket placement an "oral *infection* control" consultation should be made with an informed and competent professional for scaling root planing, comprehensive charting, continually supportive oral hygiene instruction and the application of a labial fluoride varnish. The bold but appropriate word "infection" should be used instead of trivializing euphemisms, e.g. "inflamed" or "a little swollen".

During all orthodontic treatment with fixed appliances fluoride therapy should continue every 3-6 months depending on the degree of infection and fluoride varnish should be reapplied every 6 months around the bracket perimeters. There is little need to remove archwires if the treating professional is well trained and experience. Generally, taking off archwires and replacing them just for oral hygiene prophylaxis or oral hygiene method instruction is inconvenient to patients and interferes with compliance. Usually a bleeding index, (percentage of sulci which bleed upon 20-25 gm probing) of 25% or less will keep patients safe. Subtracting this percentage from 100 gives the patients a meaningful quantization of "disease control" similar to that used for patient modulation in diabetes mellitus (serum glucose levels) or hypertension (blood pressure). This quantization enhances compliance greatly.

One should beware of token efforts in the treatment of gingival and periodontal disease in orthodontic patients. When disingenuous or modest efforts are made in this regard the deeper infection can be "covered up" and made more insidious. When inadequate or trivializing advice is given to patients "disease masking" occurs and the infection can threaten permanent bone loss by the inattentive scaling and root planing or negligent supervision. (Greenwell, 1998) Thus, supervision of gingival infection must accompany all phases of orthodontic therapy and retention. Greenwell, both an attorney and periodontist by training, integrated the scientific facts with social and ethical imperatives very well in his 2008 publication. While his ideas are somewhat doctrinaire, they reflect a significant consensus among experts in the field. He stated,

> Using adjunctive agents to temporarily hide inflammation has been termed disease masking. Disease masking should be avoided. This occurs when treatment is directed at resolving soft tissue inflammation rather than focusing on the elimination of etiologic agents form the root surface by mechanical treatment [scaling and root planing]. The use of adjunctive agents, such as antimicrobials, antibiotics, or host-modulating agents, to reduce soft tissue inflammation prevents the periodontal therapist from identifying sites that need additional mechanical therapy. Treatment is not provided and disease progression continues. The effect of adjunctive agents often lasts only while they are in use, or for a limited period after they are discontinued. Disease returns when the agent is removed because the root surface problem was never eliminated. Therefore, the use of adjunctive agents during active periodontal therapy is not recommended. (Greenwell, 2008)

The marginally doctrinaire position of Greenwell is justified because he wants no chemical to interfere with critical diagnostic feedback data (residual inflammation) which will help modulate the efficacy of standard periodontal treatment*. This view is tempered somewhat by the synthesis of Blanchard which integrates both traditional mechanical care and new chemotherapies.

> The use of topically applied and systemically delivered antimicrobial agents in addition to modulation of the host inflammatory response would be a part of the treatment strategy for patients with aggressive forms of periodontal diseases or those who otherwise fail to respond to mechanical forms of therapy. Disruption and reduction of the subgingival biofilms by root debridement are crucial for optimal effectiveness of adjunctive antimicrobial or host modulation therapy. (Blanchard, 2008)

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* Henry Greenwell DMD, MSD, JD Professor and Chairman, Department of Periodontics, University of Louisville, Louisville, KY USE (Personal communication, 2010)

For periodontal health in orthodontic patients, a meaningful and predictable compliance goal has been estimated at about 2/3 for adolescent patients by Boyd and other recognized authorities in the field. Specifically Boyd states, ""Even with a highly structured preventative program in place during orthodontic retreatment with fixed appliances, 20 to 30 % of adolescents will have unacceptable hygiene and gingivitis."* Others add that an increased bacterial burden occurs in every uninstructed orthodontic patient and ultimately creates subtle but irreversible damage in as many as 60-65% of our cases. (Waldrop, 2008) This is a particularly important statistic for surgical alveolar orthopedic patients and should be heeded with sobriety and respect. Also, a prevalence of periodontitis (attachment loss) in minority adolescents has been reported as high as 25%. (Cappelli, 1994) Yet, even localized aggressive periodontitis, (erroneously considered by some as untreatable), can be cured in 60% of patients for 14 years or more. (Mros, 2010). This is why the recent decision of the American Board of Orthodontists requires that periodontal diagnosis and therapy accompany all adult orthodontic cases submitted for board certification. Given the pernicious systemic threat to cardiovascular health (Tonetti, 2007), logic demands that this standard should be applied to children as well.

Bone loss (technically, attachment loss) is the hallmark of periodontitis and depending on the pattern of destruction may not be amenable to regeneration, due to the patient's individual biologic capacity for regeneration, preference, or personal compliance. Individual patients can demonstrate sudden bone damage during orthodontic care so periodontal probing as illustrated in Figure 9 should be scheduled every 6 months, preferably *not* by a preoccupied orthodontist. Probing error is 1mm so inter-examiner calibration is needed periodically. For the busy practitioner, a valid screening by probing only interproximal surfaces takes less than 2 minutes and can be delegated efficiently.

* Dr. Robert Boyd, Professor and Chairman, Department of Orthodontics, University of the Pacific, San Francisco, CA USA. (Personal communication, 2010)

Gingival Enlargement

Orthodontists should take a collaborative approach to treatment planning to achieve periodontal health and maximal esthetics outcomes. The presence of altered passive eruption, hypertrophy, gingival fibroplasia and true attachment loss may conspire to complicate treatment in the presence of inflammation and compromise the esthetic outcomes. Note that even a pseudopocket (gingival pocket) produced by gingival enlargement is a pathological entity that can lead to attachment loss and permanent bone loss after an adolescent is dismissed from orthodontic therapy. While the pseudopocket, by definition, does not involve attachment loss *per se*, the orthodontic-induced gingival enlargement can serve as the "first falling domino" in a cascade of pathological events and the *sine qua non* of causal connections.

Three causes of gingival enlargement can be identified: (1) the first is simple infection-induced edema, a reversible condition in short term circumstances. But the edematous condition over time leads inevitably to (2) gingival fibrosis which is permanent and often referred to as gingival hyperplasia. This fibrosis is characterized by excessive growth of connective tissue and a decrease in cellular tissue components. It is similar to, but not identical with, the pharmacologically induced gingival hyperplasia caused by phenytoin (Dilantin®) therapy. (3) Altered or frankly arrested passive eruption is a third and most serious component to gingival enlargement often seen upon debonding. While Orban and Gottlieb (1933) classically considered passive eruption a pathological entity (Fiorellini, 2006) at the time they were contrasting it with the discredited concept of physiologic recession, a notion that all apical migration of gingival tissue is normal. In the growing child and adolescent patient passive eruption is normal and physiologic, as long as the final position of the

apical extent of the junctional epithelium (epithelial attachment) terminates at the cemento-enamel junction (Bosshardt, 2005). (Fig. 1)

We hypothesize: in some cases by restricting movement of a tooth during orthodontic treatment with a fixed appliance until the course of full root development is extinguished, the stimuli of natural eruption are interrupted; this arrests passive eruption of the gingival margin and the alveolar crest. (Fig. 11B). Reflection of a mucoperiosteal flap to reduce gingival enlargement in "gummy smiles" will reveal this abnormally coronal alveolar osseous crest. (Waldrop, 2008). This is why a simple gingivectomy or gingivoplasty for ostensible redundant tissue is ill-conceived; the surgery impinges on and often inadequately reestablishes the tooth-gingiva "biological width", an anatomical region critical for attachment level stability. Of course if the tissue is truly "redundant" there is no harm; but where a combination of all three factors contributes to the gingival enlargement one can never know the exact etiologic mechanism. By minimizing risk, the flap approach to gingival margin repositioning is the more prudent course.

The orthodontist should delegate a "post-bonding inspection" to a responsible colleague who is familiar with periodontal management or scrutinize the orthodontic patient's periodontal health during the retention period. Although the patient may not make the connection between gingival pocket formation as a teenager and bone loss in the late 20's -30's, the ethical obligation remains with the referring doctor, the orthodontist and the periodontist to maintain periodontal and gingival health as good as possible during and after fixed appliance therapy. Increased public awareness and the introduction of clear aligners have helped reduce iatrogenic risk in the child and adolescent in recent years. But at the very least the orthodontist has both an ethical and legal duty to inform the patient of the risks. Then the proximate cause of any attachment loss transfers to the patient's negligence, an error of omission, in not following the doctors' directions. But third party payers can complicate this.

The usurpation of traditional standards by third party commercial interlopers has often served to homogenize protocols for better or worse. Some standards have been compromised to limited therapeutic objectives, e.g. level and alignment *only*. However, in regards to periodontal complications, some advocates have actually raised professional standards through a kind of patient "collective bargaining" with the specialty. As an example of constantly elevated standards, some American managed care companies have required orthodontists to record periodontal and gingival pocket depth before, during, and after orthodontic therapy, in children, adolescents, and adults. This antedated even the 2009 policy of the American Board of Orthodontics under the visionary leadership of Dr. John Grubb, which requires a full periodontal charting and radiographic diagnostic series for patients 18 years or older, in order to pass the examination for board certification. The incursion of corporate entities into traditional diagnostic and treatment prerogatives of orthodontists under the questionable aegis of a "corporate model" can hardly be criticized when such forward thinking policies are established by the managed care stakeholders. But such standards should be initiated by orthodontists, not corporations.

Importantly, the emergence of third party managed care and oversight managers may *not* necessarily vitiate the fiduciary responsibilities of the treating doctor. Ultimately the doctor is still responsible to the patient not a separate third party advocate. So, in cases of poor patient cooperation, a note of patient noncompliance should always be included in progress notes. Quite often patients project blame onto the orthodontist after ignoring advice and suffering untoward events. A simple "ADA" (against doctor's advice) in the progress notes can preclude a plethora of misinterpretations and unjustified recrimination. This admonition serves to clarify the often murky doctor-patient relationship in discussions, where strident claims of consumers "rights" conspicuously omit imperatives of concomitant consumers "responsibilities". Professional liability experts will acknowledge that society

should not indemnify a orthodontic patient's disappointments. But this rational point if often ignored by malcontents, third party indemnifiers, and irrational patients. A constant balance between private interests and the larger social responsibilities are defined best when articulated *prior* to all treatment.

As alluded to above, a recent landmark study Waldrop et.al. (2008) looked at the prevalence of gingival enlargement, (altered passive eruption, hypertrophy, and hyperplasia) a harbinger of pocket formation in orthodontic patients. The researchers studied the same patients 5 years after treatment, and noted the need for periodontal plastic surgery and esthetic crown lengthening. They discovered over 60% of treated patients had inferior smiles due to gingival enlargement. (Fig. 10). Enhancing orthodontic results and providing the patient with maximal esthetic results can be achieved through the control of inflammation during orthodontic treatment. Where that fails to completely secure the gingival margin at the CEJ of all teeth, surgical correction should be employed with a flap procedure. (Figure 11). This imperative is evidence-based, not simply anecdotal or conjectural; it is empirical fact.

Some doctors may use a gingivectomy (GV) to solve this problem of gingival enlargement, with hand held lasers. this is risky because few understand how ablative a GV really is. Laser therapy relies on ill-conceived notions that only soft tissue is redundant or misplaced. However if gingival enlargement is caused by altered passive eruption, cauterizing lasers can destroy biological width, and alveolar bone crests. Then pockets or recession can manifest years after debonding.

As a matter of fact, gingivectomies have generally not been widely used by enlightened periodontists for over 50 years because of these limitations and the ablative nature of the surgery. The wise

orthodontist is ill-advised to undertake such ablative surgery to "remove redundant tissue" or "take off scar tissue", without first consulting a certified periodontist familiar with the biological phenomena that orthodontic-periodontic relationships. This helps to enlighten patients of many procedural limitations. Shared management ensures shared risk. Once these salient periodontal-orthodontic issues are managed the 21st century orthodontist is competent to enter the stage of dentoalveolar tissue engineering and the burgeoning realm of stem cell therapy.

To Extract or not Extract: the Orthodontists' 100-Years War

No issue has been historically more contentious than the century-long debate over bicuspid extraction. One reason this debate lingers is that no algorithm, formally etched on a circuit board, programmed in software, or intuitively divined in the mind of the orthodontic specialist, can elicit a 100% guarantee that the clinical outcome will be subjectively satisfactory, or complimentary to facial form, 10-20 years after treatment. This is especially true because of the psychosocial diversity of adolescents, and the unpredictable "biologic systems" that are expressed from pre-pubertal to mature phenotypes. Thus, in terms of decision theory, the choice of bicuspid extraction or non-extraction protocols is largely a "standard gamble". Moreover, indeterminate variables affecting biological systems are myriad, accounting for not only the long survival of mammals through eons of natural selection compounded but also the flux of personal esthetic values that must be factored into the forecasting equation; the ability to predict ultimate outcome satisfaction approaches zero.

The problems then is not necessarily which teeth should be extracted to gain room in arch length-tooth size discrepancies. The ultimate problems are whether an unexpected flattened profile will emerge prematurely over the following in 10-20 years, given the unpredictable nature of facial growth. In such cases normative forecasting robustness in fields of future uncertainly is limited and meaningful, algorithmic prediction for individuals through time becomes less helpful than a coin toss. It seems prudent then, to apply the heuristic flexibility of Pascal's Wager. If one, in retrospect,

regrets the decision to extract in a significant number of cases, one's thinking should change. The clinical dilemma then becomes one of tissue sufficiency, *viz*. there must be enough bone mass or a potential for bone mass growth, e.g. labial to mandibular incisors when the dental arch moves facially. The answer to this question lies in the potential of genetic expression. That is, will the bone follow the incisors labially over time or will the incisors move off the bony housing with gingival recession?

As many have claimed the correlation of OTM to gingival recession is so low as to be useless in prediction (Joss-Vassalli, 2010 Djeu, 2002,) and movement in a *milieu* of specific gingival infections appears to be the proximate cause, not movement *per se*. The solution to this decisional dilemma is to create more labial bone that responds to tension gradients of root movement. That solution is now available to orthodontists in the form of minor dentoalveolar surgery. If Periodontally Accelerated Osteogenic Orthodontic (PAOO) protocols are precisely followed, labial bone can be developed (Fig. 17) and apparently even more bone can be "grown" when stem cells or viable cell matrices are used instead of "dead bone", in a demineralized bone matrices (DBM)*. (Gonshor, 2010).

Thus, labial movement may be associated with bony dehiscence in some cases of biomechanical force but stem cells can respond to force, in the labial periodontium, by forming more bone. That natural proclivity offers many patients a less destructive treatment alternative. From a risk management perspective, given that the ultimate effects of extraction therapy for some individuals is unknowable exactly, stem cell bone augmentation *via* PAOO, delivers a practical, positive alternative that can be offered to patients with impunity. While retrogressive trends or autocratic dogma may be offended, giving this alternative is a just and empowering way to share the risk of future regret with patients and a welcome reconciliation of the vexatious extraction debate.

Without a preemptive approach to minimizing peri-orthodontic infection, new horizons will be clouded by complications. But with a bold approach to clinical infection control, an understanding of bacteriology and chronic infection management, tissue engineering and stem cell therapy, new vistas

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Also known as DFDBA, demineralized freeze-dried bone allograft

are expanded far beyond the dreams of our teachers. Yet, it is up to each individual orthodontist to *choose* to explore these new frontiers or rest upon prior dogma alone. Clearly, with new cell biologies, dogma based on old notions of tissue limitations, is fated for obsolescence. *Quo vadis*?

21st Century "NewThink": The Age of the Stem Cell

In the last decade, professional societies and international academic workshops have witnessed advancements in both computer science and telecommunications which, conjoined, open spectacular vistas for the entire human species in a kind of "perfect storm"* of intellectual growth. For example, researchers at the J. Craig Venter Institute (JVCI) ** have synthesized a new species, *Mycoplasma mycoides*, from a synthetic genome. The new cells have exhibited life properties such as expected phenotype structure and behavior. Most importantly the artificial cells are capable of continuous self-replication, the hallmark of Life itself. In other words, the JCVI scientists took "dead body parts" from cell cultures, arranged chemicals in a specific order, then implanted them in a "bag of protoplasm" which then "came to life".

Using such historic opportunities, literally the dream of Mankind, a new generation of orthodontists is positioned perfectly to become the specialists of facial tissue engineering, carrying on the legacy bequeathed to us by selfless educators and corporate financial patrons. It is within this vision that the proposals herein are posited, the protocols are explained and a new legacy is dedicated. This "NewThink" readily available to all orthodontists globally, should not

categorically replace “OldThink”, but rather, refine and build upon previous conceptual basis of the orthodontic specialty for the younger generation.

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* A “perfect storm” is a metaphor for an emergent event in a mathematically chaotic or complex progression.
** www.jcvi.org

Definitions

Some definitions are in order however, lest the benefits of this protocol are misappropriated or confounded with other less efficacious techniques, an unfortunate but increasingly common event. Surface markers, *viz.* antigenic determinates, on the cell distinguish one cell type from another. But distinctions can be rather arbitrary within a continuous spectrum of maturation, making clear demarcation nearly impossible. But simply put, stem cells, (here: adult stem cells or “ASC”), are generally defined as primitive cells that mature into specific-function cells.

Specifically for our purposes, hMSC are components of the viable cell-allograft discussed in this chapter, combined with other types of bone forming cells in a processed bone matrix. The collection of precursor cells in the matrix provide many types of potentials which in fact contribute to a collective “stem cell” function.While the definitions below are generally accepted, stem cell therapy (SCT) is young and definitions may take on new connotations in the future. So the astude clinician should be intellectually vigilant.

Even with this ability of the nascent science to elicit differentiation, de-differentiation and even trans- differentiation, SCT is prone to misuse. Misinterpretation or conflation of words and ideas is common. For example, multipotential and pluripotential are often informally interchanged, not correctly, but inadvertently and out of context. This caveat will be observed and respected in this treatise but the reader should be alert to nuances and subtle distinctions of meaning. The term “stem cell”, like the words “love” and “nice” has, unfortunately been so over-used that it has lost

meaning.. In one strict sense the only "true" stem cell is the fertilized-ovum or one subjected to parthenogenesis. And more informally, a pre-osteoblast is in a sense, a "stem cell" because it can differentiate further into a mature osteoblast. Yet one must make a distinction between stem cells that undergo maturation or development and those which can give rise to more daughter cells and differentiated forms. Alas, biology in contrast to the strict sister sciences of chemistry and physics, "the only constant in biology is inconsistency". Given this flux of natural and syntactical affairs, for our purposes, the terms of "stem cell" therapy are used thusly:

Totipotent is the ability to give rise to all the cell types of the body *plus* all of the cell types that make up the extraembryonic tissues such as the placenta.

Pluripotent is the ability to give rise to all of the various cell types of the body *except* extra-embryonic tissues such as placenta components.

Multipotent is the ability to develop into more than one cell type of the body.

Stem cells are cells with the ability to divide to produce a fully functional mature cell capable of specific functions in tissue. Generally stem cells have the ability to divide to give rise to both daughter cells and more specialized function cells. In contrast, osteocytes and fibroblasts do not change into more specialized cells naturally. Stem Cell Therapy (SCT) can be local or systemic.

Mesenchymal stem cells (MSC) are non-blood adult stem cells from a variety of tissues. Although it is not clear that MSCs from different tissues are the same, they are multipotent for mesenchymal tissues, derived embyologically from mesoderm. hMSC are human MSC.

Osteoprogenitor cells (OPC) are cells dedicated to producing osteoblasts but with more surface markers that allow them to be distinguished from MSCs.

Surgically-facilitated orthodontic tooth movement (SFOMT) refers to alveolar surgery that achieves an "optimal response" to orthodontic therapy, generally by selective decortication and grafting.

Background and Rationale

Stem cell therapy rests upon a method of alveolar bone preparation that induces a temporary, reversible, non-pathologic osteopenic state. This is a temporary reduction of the organic and mineral content of the alveolus around the moving tooth root. Generally this alveolus manipulation will safely ensure that the tooth moves 200-400% faster than conventional orthodontic methods with more stability and less infection. The exact nature of histological, cytological and intracellular orthopedic effects of SFOTM, deep in the alveolus remained enigmatic until controlled studies of tissue behavior appeared in the 1980's at Loma Linda University.

Further validations of historical anecdotal claims of efficacy were added at the beginning of the 21st century and continue to emerge in the osteology literature. These critical data and further science were popularized by the prodigious collaboration of Professors Wilcko and Ferguson at Case Western Reserve, Boston, and St. Louis Universities. (Wilcko, 2003).Yet, with their copious and meticulous case study data, the intrepid Professors Wilcko did more than merely popularize SFOTM or selective alveolar decortication (SAD), a kind of superficial "corticotomy" limited to certain areas of the alveolus. By adding a bone graft to the SAD prepared alveolus, (PAOO/AOO) * they ingeniously extended the scope of orthodontics into the world of clinical tissue engineering by demonstrating how to redesign alveolar bone phenotype. We translate this legacy into the science of human mesenchymal stem cell (hMSC) therapy merging the clinical bio-mechanics of SFOTM with the mechano-biologics of modern osteology, clinical orthopedics, and the burgeoning filed of stem cell biology.

PAOO/AOO protocols work to reduce the need for bicuspid extraction with phenotype alteration but can also alter facial form (Figure 12) without orthognathic surgery. This is done by

epigenetic manipulation, effectively validating the tenets of Professor Moss's Functional Matrix Hypothesis (Moss, 1997a-d) That is, that the root of a tooth acts as a template or "functional matrix" for neo-morphogenesis of the alveolus bone. By the first decade of the 21st century worldwide acceptance of the Wilcko-Ferguson-Moss theses had grown so quickly that two American Universities** incorporated the entire gamut of SFOTM, including PAOO/AOO, into their standard curricula. This scholastic elevation challenged established therapies with a more cost-effective, healthier, and more benign out-patient science. Extrapolating present data and clinical impressions, what the future holds for the next generation of orthodontists (should they choose it) is not merely faster, better, and safer smile design. But rather, these data promise nothing less than the intra-oral, scarless designer "face engineering".

* Periodontally Accelerated Osteogenic Orthodontics (PAOO) and Accelerated Osteogenic Orthodontics (AOO) are trademarks of Wilckodontics Inc, a proprietary orthodontic school in Eire, PA USA www.wilckodontics.com

Epistemological Issues: Choice and Clinical Styles

Slowly, as the dogma of alveolar immutability surrenders to modern concepts of phenotype plasticity the alveolus is emerging as a malleable entity requiring new theories of morphogenesis and mechano-transduction. This "bottom up" paradigm is defined by the dynamics of bone healing and genetic expression to a pre-selected form, depending on root position. (Murphy NC, 2006) This provocative "NewThink" evokes phantoms of doubt in the minds of some who defend categorical mandates for "OldThink" or routine bicuspid extraction. In contrast, more thoughtful and less doctrinaire clinicians see the logical consistency and suggest that the rationale for routine bicuspid extraction may need to be reassessed. Other biologic advantages to SFOTM and SCT are their ability to (1) reduce the risk, quantitatively and qualitatively, of bacterial damage done by a prolonged care and (2) reduce relapse of unretained fixed appliance therapy. (Little, 1988; Little, 1993, Dosanjh, 2006)

Since conventional biomechanics clearly does not alter bony phenotype conceptually or in fact, innovations of the last decade should not be discounted as novelties. They are slowly replacing

old ideas with emerging paradigms more appropriate to this so-called "Century of the Biologist". Conventional dental techniques will always have their place but cannot solely define the specialty due

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to limitations mentioned above. SCT prevents or mitigates the severity of side effects and earns a deserved place in the pantheon of legitimate care. Even modern periodontal regeneration cannot match these achievements of orthodontic SCT. Periodontal bone grafting merely re - establishes original phenotype, passively. But, orthodontic tissue engineering and "stem cell" therapy achieve permanent phenotypic change, pro-actively with viable stem cell dynamics and local immunosuppression. The challenges of molecular biology are making the study of orthodontics at one time more difficult and yet more interesting for many students and practitioners alike. Technically speaking, only blastocyst cells* are truly "stem" cells. But in this discourse, the term refers to any cell that can further differentiate into a mature osteoblast. So some contend that a better description of the graft material herein is "viable bone matrix". Regardless of semantics or the perceived difficulties SCT certainly minimizes what Professor Johnston has pejoratively referred to as the "arts and crafts" dimension of clinical practice.

In this second decade of the 21st century, the advent of safe and predictable tissue science can extend the biological frontier of each new dental students and residents even further into the exciting realm of genetic tissue engineering and the potential for *in vivo* gene therapy. The ideas in this paper are a natural extension of others' efforts, and a new synthesis of both manifest clinical need and contemporary human mesenchymal stem cell (hMSC) science. Ironically, despite the sophisticated rationale, the actual procedures necessary to attain these vaunted goals can be achieved with a simple periodontal surgery which is taught to first-year periodontology residents in the United States.

The key to success lies not in a particular material or surgical procedure, but rather in the *orchestration and timing* of traditional protocols, *viz.* simply moving teeth roots into a field of healing, but strained bone. Strained bone is different than steady-state bone which the

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* embryonic stem cells (ESC), a topic beyond the scope of this chapter

orthodontist usually encounters. *A wound recapitulates regional ontogeny* and that ontogeny responds to local environmental (epigenetic) perturbations, e.g. optimal orthodontic force. Phenotype changes *via* stem reprogrammed cell differentiation. Traditionalists and skeptics may criticize SCT as unnecessarily morbid. However, considering the ablative nature of extraction "surgery" and its deforming effects on facial form, the minor side effects of rudimentary, superficial periodontal surgery (1-2 mm beneath the mucosal surface) compare most favorably.

These young ideas, once they achieve a kind of "critical mass", may eventually evolve to the point where more focus is placed on the underlying bone than the "sacred cows" of tooth alignment. This over-emphasis of novel science or the categorical vilification of extraction therapy would be unfortunate because new ideas are used best when they embellish and refine old concepts, not summarily dismiss them. Epistemological challenge must be embraced as well but often this is done reluctantly. Yet the science demands that we redefine conventional orthodontic ideas about alveolus immutability, the principle tenet by which many bicuspid extractions are justified. In light of the emerging sciences of alveolus osteology, antiquated ideas should be modified, lest our preoccupations with smile design be likened to architectural theories which focus on artistic *design* but ignore the civil *engineering* necessary to fortify a strong foundation. No sane architect would ignore the foundation of a building just because a beautiful design is requested by a client. Because, neither school, traditionalists or progressives, has a monopoly on absolute truth, new ideas refine them by dialectical collaboration, not discord.

Interestingly, this evolution of thought driving the syntheses of orthodontic tissue engineering is not the dogma of new autocratic professional authorities, but rather the sensitivity to patient preference, respect for modern dental standards, and contemporary global definitions of facial esthetics. Specifically the use of living stem cells to regenerate original phenotype damaged by infection or to change phenotypic form and mass, presents new evidenced-based and positive options to any prudent clinician who contemplates dental arch advancement or expansion. As such it is an educational imperative. (Nowzari, 2008)

Progress by Case Study Analysis

Specifically we aim to direct attention, by case study methods*, to the practicality of redesigning alveolar bone through surgical manipulation and augment the mass of available bone by moving roots into a living (viable) human mesenchymal stem cells allograft. This stem cell/graft matrix has been generally employed with notable success, in thousands of patients undergoing spinal fusion and other orthopedic surgery. (Brosky, 2009); herein we just apply it intraorally. *In vivo* stem cell therapy's strong basis in basic science (Figure 13-15) is matched only by its popularity with patients and the commercial purveyors' sensitivity to issues of safety and efficacy.

Yet, prudence dictates that final evaluations of the any orthodontic treatment should be withheld until the grafted bone is studied with radiographic documentation and/or computerized tomography; our proposal is no exception. In this regard, any absolute certitude about effects of OTM or SFOTM on the alveolus bone must be delayed 3-4 years because that is the amount of time alveolar bone needs to achieve a "steady state" equilibrium (Fuhrmann, 2002) (Figure 16)

Nonetheless, conceptual imperatives and documentation of the protocol should sustain interest in living stem cells among progressive clinicians who are already captivated by the merits of conventional alveolar tissue engineering with "dead-bone" grafts (DBM or DFDBA**) Our

* Inductive inference, leaving the second component of the scientific method, hypothetico-deductive experimentation to PhDs.
** DBM, demineralized bone matrix; DFDBA, demineralized freeze-dried bone

hypotheses, presuppose a valid "whole bone" model, that hMSC will respond more effectively to strain gradients mediated through the osteocyte syncytium as previously proposed by other research-oriented clinicians (Yokota, 2005; Zhang, 2006; Tanaka, 2006; Zhang, 2007) .

Consilience of Sciences

These "internal strain" hypotheses and "whole bone" model, discussed in previous papers (Murphy, 2006; Williams, 2008) posit the important mechano-biological notion that the alveolus osteocyte-cannaliculi syncytium *via* streaming potentials and cytoskeletal deformation in the entire alveolus (during alveolus "bone bending") are significant osteogenic events at the cell level. Both appear to act in synchrony as significant transduction mechanisms. (Ingber, 1998) This may be a universal phenomenon since these characteristics force transduction can be applied to cytoskeleton behavior in distraction osteogenesis and in many other organs. (Ingber, 2006) When teeth are moved through a healing alveolus bone graft, the resultant pressure gradients stimulate hMSC to differentiate and "reprogram" genetic expression. As wound healing recapitulates regional ontogeny, bending alveolus bone bends both DNA (Pavalko, 2003) and the protein conformations critical for morphogenesis of novel phenotypes. This explains Professor Wilcko's demonstration (Figure 17) which falsifies a common notion in periodontics, that one "…cannot grow bone on a flat surface"

In contrast, static demineralized bone or even robustly inductive rhBMP-2 used in periodontal regeneration must rely on relatively effete endogenous hMSC. Since viable cell allografts are often donated by individuals under the age of 30, their hMSC may even render a more robust potential than a patient's own bone (autografts), the presumptive "gold standard", according to some authorities. (Zaky, 2009) Moreover, relative relapse is reduced since conventional orthodontic treatment, even with extractions or circumferential fiberotomies, does not ensure orthodontic stability.

PAOO has shown better stability with equal quality (by ABO standards) and Professors Wilcko attribute this to thicker alveolar bone (Fig.17) engineered painlessly and predictably. And, stem cell allografts, so far, seem to do it faster and better.

Yet, the clinical innovation presented herein is not a pretension in basic science; it employs *translational science*, synthesizing extensive literature with practical experience to provide intellectual *gravitas* to those who wish to broaden the scope of the orthodontic specialty with compelling legitimate science. These new data, proven safe and effective, take the thinking clinician beyond the strictures of biomechanical wire-bending art to develop expertise in surgical dentofacial orthopedics, tissue engineering science and stem cell biology, (but only if chosen).

Interestingly, a viable cell allograft demonstrates less postoperative erythema and local inflammation than conventional DFDBA or decortication alone. (Fig. 12) (Gonshor, 2010) We attribute this to the pro-active nature of the stem cells and their curious "homing" mechanisms with which they "seek out" damaged tissue and suppress local inflammation. The homing behavior is evident even when cells are injected systemically but the mechanism is still elusive and remains one of the intriguing characteristics of stem cell tissue engineering.

Consilience of Style

The acronyms hMSC, OPC, Pre-osteoblasts present a conundrum for classification because viable human Mesenchymal Stem Cells (hMSC), Osteoprogenitor Cells (OPC), Osteoblasts and allografts (DBM or DFDBA) all combine, as in the case demonstrated here, to provide patients, especially those compromised by age, a veritable cocktail of safety, efficacy and styles. Investigation into government regulatory agencies by the senior author has revealed a startling degree of safety. Over 40,000 grafts viable cell allografts have been placed by clinicians with no reports of adverse reactions, immunologic rejections or graft vs. host disease. Thus, statistically speaking, it appears that the safety of this new biologic procedure exceeds that of the car which patients may drive to the clinician's office.

The graft material demonstrated in this paper does not contain a 100% hMSC population, so many clinicians and scientists feel the term "stem cell allograft" is misleading and prefer to use the term "viable cell allograft". Clinically, many patients call DBM or DFDBA "cadaver bone", disparage it *per se*, and actually prefers a graft that is "alive". Thus, the reader must understand that in a nascent science, despite its compelling validity and evidence base, syntactical ambiguities are ubiquitous and only assiduous scholarship by an individual doctor can elicit exact meaning sufficient to develop each individual philosophy and practice style. Patients are, in their infinite variability of preferences, no different. Thus, modern orthodontics must embrace anyone who is biologically well informed.

Despite this taxonomic conundrum, the fact remains that the modern grafts demonstrated herein have been tested to show that, a *minimum* of 250,000 viable bone forming cells per mL are available for healing. This does not count daughter cell proliferation and, since healing is a tissue-forming-cell "numbers game", more cells means better and faster healing. Moreover, all stem cell grafts are tested for cell count, osteogenic potential and viability. While technically, each commercialized mL of graft cannot be tested for its osteoinductive capacity, examinations for the "Three Golden O's", osteo-*conductive*, osteo-*inductive* and osteo-*genic* are made of each lot randomly.

The specific osteoinductive potential of most grafts is arrived at deductively from the very nature of DFDBA as demonstrated decades ago. Reasons for SCT success also rest on logical scrutiny. Indeed, employing the strict epistemological test of John Stuart Mill, while part of the whole, e.g. rhBMP-2, may be necessary for bone growth, none is sufficient. SCT replicates the *whole* natural phenomena more comprehensively than adding elements here and there, as an educated trial and error approach. SCT puts 250,000 "bone forming factories" in the wound not just a few ingredients. This is tantamount to "putting more cooks in the kitchen" instead of just "buying more cooking ingredients". Probably best expressed, only a unique, natural orchestration of unique healing elements, a therapeutic Holy Grail for each individual patient, is the key to success. Stem cells "read" what the local environment tells them to become. So each cell seeks and secures its own bony fate. Like a symphony, all musical components are, individually and collectively, necessary, but none are sufficient without a score. The stem cell in this analogy is the conductor, making it self-sufficient.

When patients' own bone grafts are used to augment alveolus bone mass lost to infection or to enhance alveolar development, the graft is termed *autograft*. In contrast, an *allograft* is tissue from another individual of the same species. This evokes issues of immunology. Because of the embryologically primitive nature of the hMSC, rejection and graft-host disease has not been noted despite the tens of thousands of cases in which hMSC allografts have been used. In fact there is even some evidence that the hMSC itself may help regulate activity of recipients' T-cells subtypes and antibody production by B-cells, and immune tolerance of allogeneic transplants. (Patel, 2008)

So, theoretically, one may ponder whether there would be less chance of an immunologic reaction from hMSC than more poorly processed allografts alone, which lack proactive immune response suppression. Experts generally acknowledge that one of the remarkable attributes of hMSC is that they express neither human leukocyte antigen (HLA) Class II markers, nor the

accessory molecules (CD40, CD80, CD86) necessary to activate a cellular immune response. This is why graft-versus-host disease is not an issue and why donor-recipient matching for these cells is *not required*. Thus, hMSC and viable cell allografts appear as safe as they are effective. hMSCs, we posit, may also be the preferred for PAOO/AOO because late adolescent or adult patients' have *endogenous stem cells can be recruited* by the hMSC graft. (Fig. 13)

A contrast should be made between marrow hematopoietic cells, which are removed from the graft during the fabrication process and the bone forming cells that remain. A recent report of a angiomyeloproliferative complication has justly elicited some concern among clinical neophytes in this clinical area but deeper analysis of the single report reveals that the complication of mass lesions was the result of direct injection of hematopoietic stem cells into a kidney and soft tissues. (Thirabanjasak, 2010)

Since the healing potential, and more importantly the degree of stromal regeneration, is related to the concentration of stem cells in the wound, local augmentation of stem cells is entirely compatible with other bone grafts and regenerative materials, e.g. autograft, osteo-conductive extenders, DFDBA, recombinant human bone morphogenetic protein (rhBMP-2). However, there is no compelling evidence that so called "extenders" or 'enhancers" that provide extra mass or biochemical supplements are necessary. In the absence of data to the contrary, the hMSC graft may be considered singularly sufficient and proffering accommodation to many practice styles.

Consilience of Cognition

Given this new science, we propose that it is no longer fitting to present only outdated paradigms from the early 1900's, which have been sanctified by overuse, not logical scrutiny. Popularity does not equate with rationality. Admittedly, the convenient pressure-tension model of Oppenheim and Schwartz, circa 1905-1911, might work well as simplistic fictions to satisfy

curious but uninitiated patients. But the periodontal ligament and spongiosa of bone are complex visco-elastic gels, so pressure, as in any closed hydraulic fluid system, is more or less distributed evenly throughout the phase while stress and strains are multidirectional and variable in magnitude.

Moreover, old pressure-tension models and their derivatives do not subsume either streaming potentials or alveolus bone bending phenomena wherein *shear vectors* may define the operative transduction element better than hyalinization and vascular infarction. Therefore, we suggest that in the professional lexicon of contemporary orthodontic theory we should speak in terms of "fields of multi-directional strain gradients" rather than a convenient but antiquated "pressure" and "tension" construct. As Professor Baumrind taught us, the latter ignores the environment of an anisotropic complex gel that defines the periodontal ligament and spongiosa. In this physical system, trabeculae act as "inclusions" which our larger context assumes and old dogma ignores.

In a postmodern era where "best practices" guidelines and "comparative outcome analysis" are imposed on the doctors' traditional fiduciary responsibilities, individual patient choice and welfare trumps generalities. That is to say, given that all have access to sufficient information, the ultimate arbiter of care is a well-informed patient and the best advisor, experienced to a reasoned mind and relying on intuitive but legitimate Bayesian logic, (not merely Gaussian frequency distributions) is the treating doctor. When general guidelines affect all individuals they are not dysfunctional Draconian impediments; they must be noticed and heeded. Individual and inviolable styles of legitimate cognition however, must be distinguished and exempted from larger categorical imperatives.

Regulatory Imperatives

Despite the dramatic effects of hMSC *in vivo* it is important to realize on a practical level that the human cellular bone matrix must conform to extremely rigorous standards promulgated by the United States Food and Drug Administration (USFDA) (21 CFR part 1271) and should be used only with a sterile (aseptic) technique. Regardless of the commercial source, the best formulation for carrying stem cells to the surgical site is in cryopreserved cancellous fragments, viable cancellous matrix and ground bone. During preparation the hMSC exhibit the rather curious ability to physically stick to the sides of plastic flasks. This mundane characteristic is the mechanism that allows the separation of hemopoietic elements from active bone forming cells in the donor bone marrow. However, the best test of a stem cell graft is its ability to differentiate, *in situ,* to any cell of mesenchymal origin and, ultimately, its organization, indistinguishable from the native architecture. To ensure this individual success, regulation of universal characteristics and protocols must be followed.

Criteria for hMSC graft donation

All hMSC donors must be screened to eliminate the chance of communicable disease agents. In fact leading purveyors of stem cell allografts are so strict with regulatory compliance that their testing exceeds requirement set by the United States Food and Drug Administration (USFDA) and the American Association of Tissue Banks (AATB). For example the best laboratories will commonly exclude donors who have had any xenograft or *even cohabited with a xenograft recipient*. In addition, tests for the following methodologies should be negative or non-reactive HIV 1 and 2 Antibody, Hepatitis C virus antibody and B-surface antigen or B core antibody, syphilis rapid plasma reagin or Treponemal Specific Assay, human T-Cell lynphotropic Virus type I and II antibody, and HIV/HCV (NAT) nucleic Acid Test.

The tests must be performed by a laboratory certified to test on human specimens under

the Clinical Laboratory Improvement Amendment of 1988, and licensed by the USFDA and an HCT/P testing facility. Even test kits must be approved by the USFDA and records of testing are maintained for future consultation. Donor eligibility is regulated by the USFDA and screening includes assessment of both medical and social history as well as physical examination. And, quality testing of each lot for osteoinductivity and cell viability is also part of USFDA records.

Tissue recovery techniques

Recovery or "harvest" of the bone graft is performed by licensed tissue bank personnel using aseptic techniques. Records review is collected at the time of recovery and reviewed again as part of the donor eligibility determination by the company selling the hMSC graft. In the case study presented here (Patient E.O., Figure 3-6) an hMSC graft, strict processing standards were confirmed. After harvest from a selected donor, the graft was processed in a proprietary manner that included disinfection with povidone iodine, cryopreservation solution, plasmalyte parenteral electrolyte/mineral combination, dimethyl sulfoxide (DMSO) and human serum albumin. Antibiotic solutions included Gentamicin sulfate, vancomycin, amphotericin B, Dulbecco's Modified Eagle's Medium (DMEM; low glucose, with phenol).The graft was treated with enzymatic solution, sterile grade enzyme, phosphate buffered saline (PBS) and processed with hydrochloric acid, hydrogen peroxide and PBS. As a fail-safe redundancy the MSC graft manufacturer* physically inspected a random sample of each lot to test for destructive micro-biological testing and ensure that the results show "no growth" after 14 day incubation and each lot was tested with an *in vitro* assay for of viable osteogenic cells and osteoinductive potential. (Fig. 29)

Surgical Protocol

Preparation

The following guidelines were used in the present case example. After the patient has been interviewed, screened for surgery, co-signed and initialed a 4-page informed consent, sedated and anesthetized with block injections and local infiltration, the patient is draped for the surgical procedure. Surgery commences immediately after all brackets are secured on the teeth to be treated. Surgery is usually limited to only those areas where orthodontic tooth movement is compromised by insufficient alveolar bone. This insufficiency sometimes can be visualized on

* Nuvasive, Inc., Diego, CA USA

3D replicates of computerize tomographs but the density of dentin and thin alveolar bone often obscure the exact location of the alveolar crest even with modern imaging machines. This obscurity itself may be used as an indication for stem cell allograft augmentation. In our protocol arch wires are placed immediately after the last suture is secured so standard OTM can immediately begin stimulating the stem cells by straining the graft. This is the critical epigenetic perturbation that creates the new phenotype as the surgery overcomes tissue stability or "epigenetic buffering". (Fig. 27) Once this is fully conceptualized by the clinician, periodic transmucosal perturbations can extend the time of induced osteopenia, *viz.* the regional acceleratory phenomenon (RAP).

Graft Delivery

After the hMSC is removed from cold storage, it is immediately placed in a sterile saline bath at 37 ± 2^{o} Celsius (95^{o} -102^{o} Fahrenheit) to thaw slowly over 15-20 minutes. This temperature must not be exceeded otherwise the cell viability is compromised. After the graft is thawed according to the manufacturer's protocol, the cells will maintain viability up to 2 hrs post-thaw when left in its cryopreservative and up to 6 hrs post-thaw when cryopreservative is decanted and replaced with sterile saline or 5% dextrose lactated Ringer's Solution (D5LR). Some dubious political

regulations (e.g. New York State) demand that the graft be used within 4 hours, even though scientific data shows longer cell viability. What makes this viable allograft interesting and valuable is the fact that it can be stored for future use and simply defrosted like a bag of peas. The shelf life, of course, is greatly influenced as a function of temperature. However, stored between -45 and -75 C the cells maintain viability for 90 days. Stored at -80 C the cells will remain alive for *5 years!*

Sometimes in practical clinical circumstances delays are encountered. This is not a serious problem with he hMSC grafting because the graft is viable for 2-4 hours. However it should be noted that if the 2 hour delay is exceeded, extra quantities of saline do not necessarily ensure extra cell viability. After the graft has settled to the bottom of the liquid preservative and prior to use, the supernatant liquid cryopreservative (decant) is carefully discarded. The graft then moves freely upon inversion of the container when ready for transfer to the patient. (Figure 20).

In medical orthopedic cases, the antibiotic concentrations associated with bone cements may have skeletal cell toxicity above certain thresholds. This can be seen *in vitro* by noting differences in cellular morphology. For example, cells exposed to ciprofloxacin may considerable changes in spread, cell membrane, and extensions. Antoci (2007) found that ciprofloxacin doses greater than 100 µg/mL and vancomycin or tobramycin doses greater than 2000 µg/mL can severely decrease cell proliferation. In our particular case a modification of manufacturers' protocol was made. Because of our septic field, a final Clindamycin lavage is done immediately prior to graft placement. Then the excess Clindamycin is poured off the graft leaving only the Clincamycin-soaked hMSC ready for placement

Our modification, a quick 15 second antibiotic lavage is commonly employed with out-patient periodontal regeneration using demineralized bone matrix (DBM/DFDBA) to reduce bacterial contamination. Post-operative histological analysis confirms that the Clindamycin rinse is safe. (Fig. 32) It should be noted that this departure from manufacturers' instructions may theoretically reduce cell viability but there are no controlled studies to prove this point. Striking a therapeutic balance between antimicrobial effects and hMSC toxicity is a profound question that must be a part of clinical heuristics for each practitioner in the absence of compelling controlled studies. Our experience suggests the theoretical threat may not be a practical consideration in the periodontal surgical environment but future studies would be helpful to the clinician and edifying to the science.

If antibiotic solutions are diluted adverse effects may be minimized but at the present time medical protocols in operating room environments usually substitute the antibiotic rinse with a final lavage of dextrose 5%, lactated Ringers solution (D5LR). This is the manufacturers' recommendation and the merits of our antibiotic lavage are admittedly debatable for some.

For a prudent clinician, in the absence of controlled studies, either using D5LR or standard concentrations of liquid Clincamycin (150mg/mL) diluted 50% with saline before mixing with the MSC may be the best course of action. Future studies should clarify these vagaries.

Surgical Flap Design and Management *

Sub-marginal incisions with AOO may be helpful to preclude unaesthetic embrasure opening. However, when periodontitis presents, PAOO is employed and sulcular incisions should be used as was done in our case studies. Surgical flap design guidelines are general and can be somewhat modified by patient preferences and each surgeon's objectives, experience, and style. Our initial incisions are made, 1-2 teeth, mesial and distal to the graft site, creating an envelope full-thickness (mucoperiosteal) surgical flap. The flaps are reflected for inspection of the labial and lingual alveolar cortices and vertical tension-releasing incisions are made where necessary at the

end of the sulcular incision. This allows coronal positioning of the flap without tension or graft spillage. Maintaining grafts distant from the vertical tension releasing incisions also aids in stability since micro-movement of graft material may limit full engraftment, i.e. integration of the graft to the decorticated alveolus.

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* Unquestionably, the best instruction in surgical technique is provided by Professor M. Thomas Wilcko of Case Western Reserve University, in Cleveland Ohio, USA. His surgical protocol enjoys the singularly best track record and has been held to the highest standard of scientific scrutiny. The reader is seriously encouraged to take the 2 day instruction at the teaching facility in Erie, Pennsylvania, USA. www.wilckodontics.com

Because the graft increases the mass of bone covered by the flap, all tension in the replaced flap must be eliminated. A flap under tension will result in necrosis of the edge and regression to the vestibular depth of the vestibule exposing of a healing granulation mass. While, surprisingly this complication may not necessarily result in complete graft slough, the secondary intention healing may delay total engraftment. Clinically, this complication extends the healing time 3-5 times longer than that seen in primary intention healing.

Before the alveolar bone is decorticated to receive the hMSC allograft all granulation tissue (Fig. 21, arrow) and root accretions should be removed with standard periodontal debridement techniques and root planing. Then punctuate and linear decortications (about 2-3 mm are made into the spongiosa) (Fig. 22A) to liberate endogenous mesenchymal stem cells from the marrow. It is important that copious bleeding (Fig. 22B) be evident before the MSC allograft is placed on the recipient bed. Generally the traditional standard for periodontal surgery is strict hemostasis but that is encouraged for visibility during resective or ablative surgical procedures. Stem cell grafting is different. Fragile graft viability depends upon a specific nutrient source, bleeding is encouraged with hMSC. Because the rigid allograft matrix binds the hMSC graft tightly, containment in a bloody field is usually not a problem.

After sutures have been loosely placed, the graft is taken directly to the donor site from the sterile bottle with a sterile spatula and molded to the contour of the decorticated alveolar cortices (Fig. 22C) where the clinician may anticipate facial root movement.(Fig. 22D) Where the alveolus is healthy and periodontal support is not compromised simple AOO protocols can be employed with predictable success. However, when the patient presents with periodontal infection, PAOO should be the protocol of choice because changing phenotypic design is combined with standard periodontal regeneration.. This way many objectives are achieved and the patient need only recover from one surgical procedure that maximizes therapeutic goals. The alternative of separate procedures would be logically untenable as three sequential treatments, i.e. regenerative surgery, fixed appliances, and surgical phenotype remodeling. SCT achieves this more efficiently with one treatment, in less time and under one fee. This synthesis adds extra utility making PAOO and SCT valuable at any cost.

In these periodontal diseased cases (as with our case study, patient E.O.) the bases of all infrabony defects should be thoroughly decorticated prior to graft placement. Timid attempts to minimize decortication in an effort to reduce bleeding or post operative morbidity is an ill-conceived notion; comprehensive decortication will not necessarily produce more post-operative pain or edema. The decortication is rarely deeper into the spongiosa than 2-3 mm and replacement of the mucoperiosteal flap with efficient surgery is better assurance of comfortable post-operative patient comfort than ineffectual decortication. Poor technique causes pain.

The PAOO/AOO protocols are indeed technique sensitive so one must strike a rational balance between aggressive tissue manipulation and prudent restraint. For example, ecchymosis, a physiologically insignificant event commonly seen in the very elderly patients is admittedly a

psycho-social liability. Yet this can be minimized even in patients with friable integument if flap reflection is not pushed far beyond the mucogingival junction.

Loosely suturing the flap with a continuous locking suture just prior to graft placement allows rapid stem cell placement and bone coverage by providing a pouch-like recipient site. Once the allograft is secure, the flap can be tethered over the graft by simply tightening the continuous locking suture like a purse string or saddle strap. (Fig. 22D). This leaves the graft and donor site exposed no more than 3 minutes.

After the graft itself is placed firmly onto the decorticated labial bone surface the flap is sutured to its original position and secured with cyanoacrylate. (Fig.23) According to the Functional Matrix Hypothesis (Moss, 1997a-d) and recent experimental data (Cohen, 2008), as the roots move into the graft, the strain gradients and endogenous growth factors interact to stimulate hMSC differentiation (Chun, 2006) and morphogenesis (Moore, 2005) creating more bone mass and better form.

Post-Surgical Evaluation

There is no need to overfill the site, as is often recommended with standard periodontal regeneration. The need is more physiologic form, not more bone *per se*. Indeed, as the graft matures, great amounts of graft will naturally resorb as teeth are moved. Yet this is not justification for minimal grafting either. The regression and reshaping of the stem cell allograft is not a measure of failure as it would be with filling a periodontal infrabony pocket. Rather it represents a natural redefinition of form, a new phenotype engineered by the body specifically appropriate for the type of tooth movement and their final position of the dentition *vis a vis* the labial alveolar cortex.

The final mass and shape of new bone will be determined ultimately by the angle of the tooth to the alveolar labial cortex over the course of the next 2-3 years. In the anterior sextants of the dentition, a convexity formed by the graft immediately after grafting reshapes itself during healing to a specific labial concavity. The final concavity is termed a "Wilcko Curve" after its namesake Professor M. Thomas Wilcko. Surgeons new to the concepts of SCT may erroneously think that the graft is being overly resorbed as incisors are moved. They should be reassured that the curve is an important landmark in the study of this uniquely engineered bone wound healing because it redefines A-Point and B-Point. It is also an important concavity because the final curvature serves as a reliable marker of morphogenetic homeostasis. After 2-3 years maturity (Fig. 24) the anatomic form (phenotype) and clinical "success" is defined not by the amount of original bone graft *per se*, but rather the form defined by the angulation of the lower incisors to a fixed anatomic landmark.

Cell Rejuvenation with TMP

The intermittent *non-resonant* stresses that roots transfer to bone during mastication and fixed appliance activation perpetuate the osteopenic state of the regional acceleratory phenomenon (RAP) discussed in earlier papers. (Murphy, 2006; Wilcko, 2008). When patients do not have archwire adjustments every two weeks, the alveolus may lose RAP and revert to a more calcified steady state. In the practical case of patient illness or the real challenge of staff vacations lasting longer than 2 weeks some doctors may be reluctant to take on this new protocol. On a practical level, the best protocols are meaningless unless they can adapt to real-world exigencies like patient non-compliance, excessive expense, or scheduling conflicts. So some modification of the protocol must accommodate these predictable complications of therapy. The best tool for this contingency is trans-mucosal penetration (TMP) into the alveolus as an intentional, controlled and therapeutic wounding. Specifically, if the operated area threatens to decalcify, one merely drills holes, around the tooth to be moved, into the alveolus approximating the depth of the center of rotation, with irrigated high speed #2 round bur.(Fig. 26)

Reactivation of RAP and a rejuvenation of stem cell viability can be accomplished by making these punctuate penetrations directly through the alveolus without flap reflection. The acronym "TMP" represents the "Trans-Mucosal Penetration" that one sees clinically (Fig. 25). Scientifically, TMP also represents "Trans-Mucosal *Perturbation*" the event that overcomes canalization of recalcitrant tissue behavior seen in Waddington's Epigenetic Landscape. (Fig. 27)

Because orthodontic mechanotherapy may be protracted and the mechanically induced RAP lasts only 6-9 months, SCT may need to be prolonged by TMP. These TMP "boosters" are helpful epigenetic perturbation of hMSCs to re-direct their differentiation toward the desired trajectory. Note that hMSCs can produce "daughter cells" for as many as 6 generations before telomeres decrease and the stem cell is depleted of differentiation potential by natural senescence. The TMP technique presumably activates daughter cells as well; it is as simple as its illustration. A high speed surgical length #2 round bur with external irrigation, driven painlessly into the anesthetized alveolus, toward the center of rotation is effective immediately and heals quickly. Afterwards, discomfort can be satisfactorily treated with 200-400mg of ibuprofen or other standard commercial analgesics.

The TMP is repeated every 1-2 mm circumferentially around the tooth decorticating with a tunneling movement where possible. With TMP of the lingual cortex of the alveolus to facilitate rapid labial movement; incisors can be tipped "epigenetically" *in a matter of days*. (Fig. 18 A-D.).And TMP can be employed independent of PAOO/AOO surgery or hMSC grafting. For example, where late second molar eruption into a malaligned position delays debonding, cases can be finished on time with simple TMP and 2 week intervals between extrusion adjustments. (Fig 26-C).

Contraindications

The absolute number of stem cells at the site of the wound decreases as a function of age in the human and the absolute number of MSC correlates positively with the degree of regeneration potential, hence the rationale for hMSC grafts instead of simple DBM. Overall, the excellent basic science, (Figs. 13-15) the safety, efficiency, reliability of the source, (Fig. 28 & 29) reasonable cost, and patient aversion to "dead cadaver bone" all combine to make viable stem cell allografts a very promising material for PAOO and the development of orthodontic art into surgical orthopedics.

However, one must not be blind to contraindications. Contraindications for the use of stem cells are the relative age of the patient and the potential for full natural regeneration due to stem cell populations *in situ*. Specifically, these include immuno-compromised patients, vascular pathoses, uncontrolled diabetes mellitus, fever, degenerative bone disease, bone infection, osteomyelitis, pregnancy and stand-alone weight bearing sites, e.g. fremitus. This also includes patients on high does calcium supplements or bisphosphonate therapy (Fosamax®) The goal of this SCT protocol is to maintain an osteopenic state, the opposite of bisphosphonate and calcium supplements.

Clearly an absolute contraindication presents when patients are allergic to any component in the graft or antibiotic lavage. This is often indeterminate but should be included in all informed consent forms and reinforced with verbal inquiry of both the patient and responsible family agents. Signs of unanticipated allergic reaction include marked erythema at the surgical site beyond that normally seen during the immediate post operative course, cutaneous urticaria, rash, hives or laryngeal edema ("fullness", "tightening" or "constriction" feeling in the area of the patient's throat). The latter obviously demands emergency medical attention.

Practical Considerations

The graft is manufactured with the intention of single patient use only. hMSCs should never be refrozen after the initial thawing because refreezing causes intracellular crystallization and cell death. Besides killing cell viability, re-freezing or reusing the MSC graft for a second patient poses an untenable risk of cross infection and a serious ethical breach. Obviously the expiration date on the container must be respected and strict adherence to recommended storage temperature is necessary. It should be noted that common household freezing units cannot be reduced to this temperature and, because of the critical nature of the material even storage in commercial, hospital or scientific lab freezers should be monitored to eliminate the chance of temperature fluctuations.

A significant advantage to processed stem cell graft is the fact that the amount of graft by volume is virtually unlimited whereas autographs produce a paucity of graft material and require a second surgical wound at the harvest site. Also, a prolonged surgical time can cause increased pain and swelling in the postoperative course. Slow, prolonged surgery dehydrates reflected tissue and often compromises the blood supply to the graft. Consequently expedient execution of the graft surgery is encouraged and larger areas of stem cells grafts should be avoided if they require long exposure to air. This is not an absolute contraindication however because every surgeon possesses a unique style that should not be compromised if it is manifestly successful. However, stepwise sextant or single arch surgery is recommended for the neophyte.

Initial periodontal therapy, also called “hygienic therapy“ suggests that extensive wound debridement over the course of 6-8 weeks is necessary through extensive scaling and root planing. This has been taught traditionally as an important requirement for periodontal surgical success. But the rationale has become confounded over the years. The principle reasons for

initial therapy are (1) to reduce the inflammatory component of a periodontal infection so surgery time may be minimized, and (2) provide convenience and visibility to the surgical site. Additionally, some doctors claim initial therapy allows greater time for doctor-patient bonding, a not-insignificant issue.*

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* A wise adage advises, "Never operate on a stranger."

This is understandable for resective surgery and some cases of highly inflamed cases of horizontal bone loss where complete pocket elimination is attempted. So one must clearly identify infrabony defects with computerized tomography or keen clinical examination before deciding on the merits of protracted initial therapy.

However for regeneration and SCT, prolonged scaling and root planing may be counter-productive since inflammation,. Inflammation is not all bad. By its very nature, localized acute, but uninfected inflammation carries growth factors and other elements that facilitate regeneration and phenotype modification. Yet, a dilatory approach to periodontal surgery is *de rigueur* for some standardized plans and unfortunately has precluded profound regeneration in many patients.

The counterpoint suggests that highly inflamed soft tissue (in the flap) is the best recipient of graft material. Moskow (1987) showed that many forms of periodontal therapies are effective and eliminating infective elements from a wound just minutes prior to grafting may be superior to debridement over 8 weeks. The ability of acutely inflamed tissue to heal as well or better than chronically inflamed tissue has been general knowledge for over 100 years. Certainly a clean wound or so-called "cold lesion" facilitates expedient surgery. On the other hand, operating in

clean but highly inflamed tissue ("hot lesion") surfeit with growth factors has led the senior author to many startlingly successful regeneration. Nonetheless there is disagreement on this point that the reader should respect.

Doctors may disagree but one thing is clear: root surfaces should be free of debris, calculus and necrotic cementum and infrabony defects should certainly be extensively degranulated and decorticated immediately prior to graft placement. Remember that protracted initial therapy is no guarantor of SCT success.

Surgical contraindications do not differ from those connected to any standard periodontal osseous or regenerative surgery. Special attention should be made to diabetic patients who, when uncontrolled, present serious problems to the periodontal surgeon. Where the diabetic patient is controlled, most authorities agree that healing is no different than a non-diabetic patient. However, if serum glucose levels are erratic, as measured by the best test, glycosylated hemoglobin (A1C), exuberant granulation tissue may form at the graft site, demanding post operative intervention and reducing the amount of healthy engraftment. Until specific literature explicates the exact relationship between medically compromised patients and predictable clinical outcomes, the elective surgery discussed herein should be considered only upon responsible consultation with the patient's physician.

Smokers, often problematic patients for dental implant procedures, are also notoriously poor candidates for regenerative periodontal surgery and should be selected for SCT with caution.

For reasons unknown, clinical experience has indicated that empirically, smokers and erratically controlled diabetic patients seem to recover quite well from conservative, non-regenerative facilitative procedures such as TMP **(Figure 11-23).**

Corroboration

The clinical examples of stem cell therapy in dentofacial orthopedics noted here have been independently corroborated in a number of other venues. Probably the most salient of these is the excellent work of Gonshor, McAllister, et. al. (2010). In their studies of viable cell matrices in sub-antral sinus augmentations they note, as we did in our model, less histological and clinical inflammation after surgery. Biopsy specimens showed greater volumetric regeneration and denser bone when the hMSC and matrix were compared to autogenous aspirates. Moreover, Figures 32-35 demonstrate histological sections at 3-4 months that looked almost identical to ours taken from the labial cortices in strained bone at 2 months. The only difference in their protocol is that they investigated steady state bone to regenerate in a fixed phenotype; ours is a dynamically loaded model that changes the phenotype of a bone which many still erroneously consider immutable. Nonetheless, their similar but independently generated results tend to argue that our hypotheses may be universal.

Future Research Needs

Yokata and Tanaka (2005) reported that, compared to no load controls in mice, osteogenesis was markedly induced with strains approximating 30 microstrain, a strain well below the minimum effective strain of ~1000 microstrain, thought necessary to produce bone formation in *ex vivo* mouse femurs. Moreover, there seemed to be a correlation with streaming potentials and fluid flow speed in the medullary cavity of the bone. This reinforces both our theory that "bone bending" changes genetic expression (Murphy, 2006) and Baumrind's 1969 attack on the conventional pressure-tension model. Mao (2003) has also studied bone responses beyond the ligament and reported that one needs a bone strain threshold of approximately 500 microstrain for inducing sutural osteogenesis, another reasonable benchmark in the absence of other data.

Interestingly, a regiment of "20,000 microstrain, 10 times a day," has been documented for distraction osteogenesis of the mandibular corpus. (Meyer, 2004)

Despite these landmarks, more *in vivo* studies should be done to specifically elucidate the range of maximum osteogenic potential for healing alveolus bone. *In vivo* strain gauge analysis could easily calculate the exact strain that various archwires can deliver with great efficiency. This can be achieved simply by adapting the sophisticated *in vitro* Wheatstone bridge electronics developed by White (1979) and Murphy (1982).

Building upon prior reports, by Davidovitch (1980) in dentistry, and Connolly (1981) in medical orthopedics, Stark et.al. (1987) demonstrated that the application of a pulsed electromagnetic field increased both the rate and final amount of orthodontic tooth movement and found histologically greater amounts of bone in an animal study. Park, et.al. (2004) recently extended this notion with the use of more sophisticated microcircuitry. These observations derive, in part, from fundamental biological phenomena that relate ionic flux and bone healing to exogenous electrical stimulation in medical orthopedic studies of long bones. Similar interaction may also occur with electric stimuli and the hMSC in this graft model. But that relationship is not yet established. Both *in vivo* and *in vitro* studies could elucidate the relationship in greater depth.

Gonshor and McAllister (2010) argue that concentrated bone aspirates do not necessarily show results superior to standard autogenous bone grafts even when extended with a viable cell matrix. Their hypothesis is that the prepared viable cell allograft delivers cells that are concentrated more firmly fixed to the matrix, and do need to compete with non-bone precursor cells, (e.g. hematopoietic cells excluded in processing) for oxygen in the healing wound. This point is cogent and should be further investigated.

They also agree that the higher the concentration of cells produce better and faster healing. Thus the concentration of 250,000 bone precursor cells per mL in our graft far surpasses the presumptive threshold concentration of 1,000. However, the exact threshold for the alveolus is presently unknown. The work of Charras et. al. (2001, 2002) demonstrates the mechanical force elicits immediate intracellular biochemical changes that are transferred to contiguous cells. The Charras model would be most helpful in elucidating the relationship between exogenous alveolar orthopedic force and intracellular dynamics demonstrated by Ingber's (2006) research.

Little do biologists know which bench-top science project will ultimately prove useful. Since Urist (1965) first identified bone morphogenetic proteins as the critical growth factor (GF) in bone repair, the fields of molecular, cell and developmental biology have become increasingly important in dentofacial orthopedics and have defined gross anatomical landmarks and clinical guidelines with increasingly greater refinement. Specifically how GF relate to bone strain is not yet clearly defined in our model.

A.K. Staples-Hausenschild (2006) noted, through her *in vitro* investigations of hMSC cultures, that the use of growth factors like TGF-3 promotes chondrogenesis and osteogenesis. The use of GF alone however, does not allow for physiologic orthopaedic function or structural development *in vivo*. It appears that mechanical stimulus is essential for full phenotypic expression. Specifically Staples-Hausenschild writes,

> "….how hMSCs respond to different types of mechanical loading, how this response differs from a traditional growth factor approach of inducing cellular differentiation and how their responsiveness to mechanical stimulation varies with cell differentiation stage are all critical for the successful design of tissue engineering constructs that are optimally organized for a specific mechanical function." *

Because of this observation our *in vivo* model appears as a most appropriate research complement to a wealth of bench top science. Also, the model described herein has great potential because of

the practical access and the ability to take repeated samples for longitudinal developmental studies.

Recently, clear removable "aligners", compatible with both PAOO (Owen, 2001) and SCT have been presented to the dental community as a method of creating socially pleasing smiles without metal fixed appliances bonded to teeth. The gentle, intermittent biological stimulus they emit may actually provide a more biological stimulus for alveolar osteogenesis that even the "light"

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*Staples, Anne Kathryn, PhD, (2006)Mechanical and biological mechanisms of regulating human mesenchymal stem cell differentiation, a PhD dissertation, from the University of California at San Francisco and Berkeley, Advisor: Dr. Jeffrey Lotz.

forces of fixed appliances. Since clear aligners are also more hygienic than conventional fixed appliances they pose less infection risk to SCT cases. We know of no studies presently investigating clear aligners and stem cell grafts.

The ultra-low forces imparted with thin wires in brackets with great tolerances in the slots ("slop") may be more osteogenic than other conventionally-defined "light forces". The function of thin archwires jostling in the relatively low friction environments can produce biologically active 500 microstrain. The intermittent, *non-resonant* strain they deliver to the alveolus cortical plates, during diurnal function and even mild nocturnal bruxism of low magnitude might be sufficient. How much mechanical stimulation hMSC allografts "need" to form clinically superior bone is still unknown. But "buttressing bone", the labial cortical bone seen in occlusal trauma, is replete with reversal lines and layers of viable osteocytes that serve as a clue. At the very least, buttressing bone gives histological evidence that compensating osteogenesis is active beyond the labial and lingual alveolar periodontia.

It is axiomatic that not every clinical case will follow a predictable path. Bilateral asymmetrical movements, and various latent periods are the norm in all human cases to a greater or lesser degree. Even if orthodontic appliances deliver "optimal force" one must understand that individual biodiversity will render an unpredictable bone response in many cases. It is no different in SCT. Nonetheless, additional scientific knowledge of cell behavior can only fortify and improve our predictive powers and minimize both treatment outcome variance and morbidities. For example, Wong, Rabie, et.al. have reported that using intramembranous bone grafts can result in much more new bone than endochondral bone when grafted into standard skull models of experimental animal defects. (Wong, 1999; Rabie, 2000) They state,

> …in cases of ridge augmentation, ridge reconstruction and repair of large alveolar clefts, DBM_{IM} powder could augment the healing and integration of IM bone grafts, presumably more quickly than the IM graft alone. This allows earlier loading of the grafted site, leading to better remodeling and incorporation of the graft.

The bone which SCT forms is intramembranous in origin. However, this kind of subtle knowledge or its clinical application is not generally known to clinicians due to the intellectual insularity of private practice and the intellectual prejudice that often accompanies doctrinaire specialization, hence the need for open, meaningful dialogue among specialized disciplines.

Conclusions

A consilience of styles is evident among clinicians such as Damon (Murphy, 2008), Williams (2008), and Wilcko (2008) when an open-minded approach to literature interpretation is employed. Damon and Williams may merely be doing what the Professors Wilcko are doing only slower and non-surgically. The basic science seems similar. Seemingly disparate terms such as "osteogenesis at a distance", "osteoblastic recruitment", "bone matrix transportation" and "compensatory appositional osteogenesis"*, are all just disparate attempts to explain the same observed phenomena in terms of scientific principles.

While each may not capture the entire domain of reactive bone physiology, an open-minded approach and patience with clinicians' observations promotes synergistic collaboration which is the stuff of progress in any discipline. Since 1950's when Reitan refined the theories of Oppenheim (1911), Schwartz (1932), and Sandstedt (1904) orthodontists have been seeking mechanisms that explain or predict tissue behavior and only time can determine which prevail. By looking beyond the ligament and noting a "whole bone" perspective, one does not deny events in the periodontal ligament, but rather see them as part of a larger holistic system of cells

* A term attributed to Reitan and suggested as most appropriate by Professor James Burch, NOVA Southeastern University, 2008.

and organs, (the alveolus "organ" being the principle focus).

As we enter what has been called "The Century of the Biologist" many old paradigms have fallen and many more should be redefined. Even the concepts of frontal and rear resorption and PDL capillary pressures as therapeutic guides are questionable models for SCT. The idea that orthodontic force should be less than internal capillary pressure in the periodontal ligament is challenged by the observation at the cell level, capillary pressure differentials can approach zero.

Therefore, perhaps all orthodontic tooth movement has an "undermining" component, especially when we realize the periodontal ligament is only about 0.25mm wide on average, a problematic clinical adjustment standard indeed. Once the gestalt, of an engineered "host response" is fully envisioned, from intracellular cytoskeletal events to accelerated OTM, all theories and empirical data are seen as mere parts of a whole and all observers can bring something to the intellectual table.

This chapter has demonstrated good contemporary clinical science and synthesizes a new professional frontier, one readily available to earnest, young (and young-thinking) orthodontists who want to expand the scope of the specialty deeper into areas of legitimate scientific province. It is an embellishment of the traditional best. This chapter was inspired by antiquated, dry, hackneyed and prosaic text that litters intellectual fields with banal nostrums, exaggerated claims, wishful thinking. Overwrought commercial product promotions are especially ominous. As practical clinical science, orthodontics should not deny the critical role of corporate largess to scientific progress, but it is merely an efficient social engine; it is neither the driver nor the ethical roadmap. Strategies that hold markets too dear and idealism too distant ultimately devolve; stark commercialism can defeat itself. This exploration of 21st century biology hopes to counter mindless platitudes and empty phrases which march lock-step in search of ever new gadgets. Orthodontics has always appeared as a clinical art or technical discipline in search of a scientific justification. Now, modern science, albeit encumbered by some postmodern apostasies, can deliver it. Observations of functional orthopedics, viewed through prisms of cellular and molecular biology, reveals how all participants can work in harmony. With this dentofacial orthopedics, or more specifically alveolar surgical orthopedics, a new generation of orthodontists can attend to a science that even justifies the non-surgical perspectives of Burch*, Damon**, Williams*** et. al., and carries the goals of Reitan and European functional orthopedists to new frontiers at cell level biology.

The observations by Williams and Damon are particularly weighty because of the widespread corroboration by practicing clinicians, a corroboration often ignored by critics. While scientific investigations have not yet clearly defined the *in vivo mechanisms* of their work, their theses and biological *systems* (not merely the brackets or the hardware), their clinical success must be acknowledged. Professional criticism can fault the explanation of tissue mechanisms but the corroborated clinical results speak for themselves. They are not alone in their discoveries, but

merely participate of a long historical cascade, adding important pieces to a slowly evolving intellectual mosaic.

In this regard one must note that, in the interests of epistemological integrity, scientific evidence can neither disprove their hypotheses nor deny the legitimacy of their corroborated observations. Conceptually, scientific experimentation (based on logical positivism and inductive inference), even with sophisticated statistical analysis, can never prove a negative. Past attempts to disprove their observations, have merely failed to disprove null hypotheses. And the persistent rationale for non-extraction therapy by the alveolus bone adaptation, which stem cell biology amplifies, is made

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more profound. The clinical evidence of alveolus plasticity, previously documented by Hom (1984) and Kokich (2005), fuels an historical debate that cannot be denied and will not depart soon. It is the manifest responsibility of investigators to pursue further research to explain the phenomena noted by these and similar clinicians. What is needed is a sophisticated falsification approach (Williams, 2008). But, because of clinical logistics and professional ethics, that may never be possible. The best tests, multiple investigations among monozygous twins with histological block sections would be most impractical at best.

Even if animal studies were to demonstrate that alveolar plasticity is predicable in selected cohorts, the conclusions would, no doubt, be reassuring. But in view of previous compelling data, and the evidence-based work of the Ferguson-Wilcko's collaboration (2008), such studies would be appear as mere paeans to the obvious. The pressing need is to explicate *biochemical mechanisms*. For now, we must rest secure in the knowledge that for some patients, self-selected from a vastly heterogeneous and enigmatic statistical universe, many treatment styles appear to be helpful to

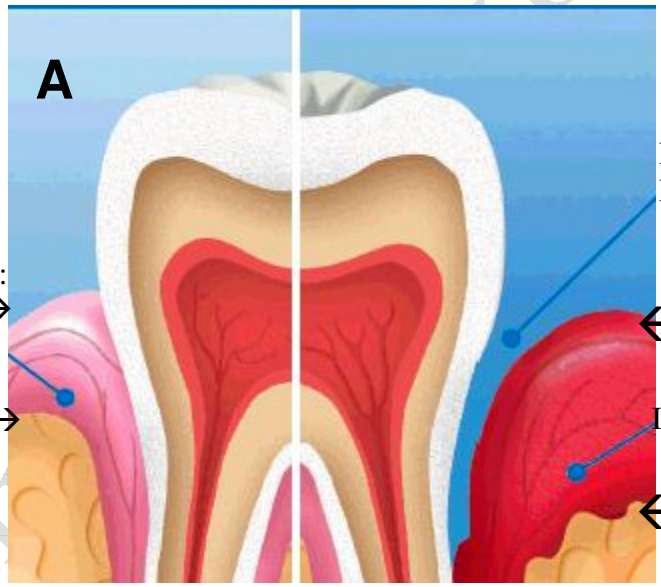
some patients despite the elusive universal laws of orthodontic therapy that we all wistfully, but unrealistically, dream of.

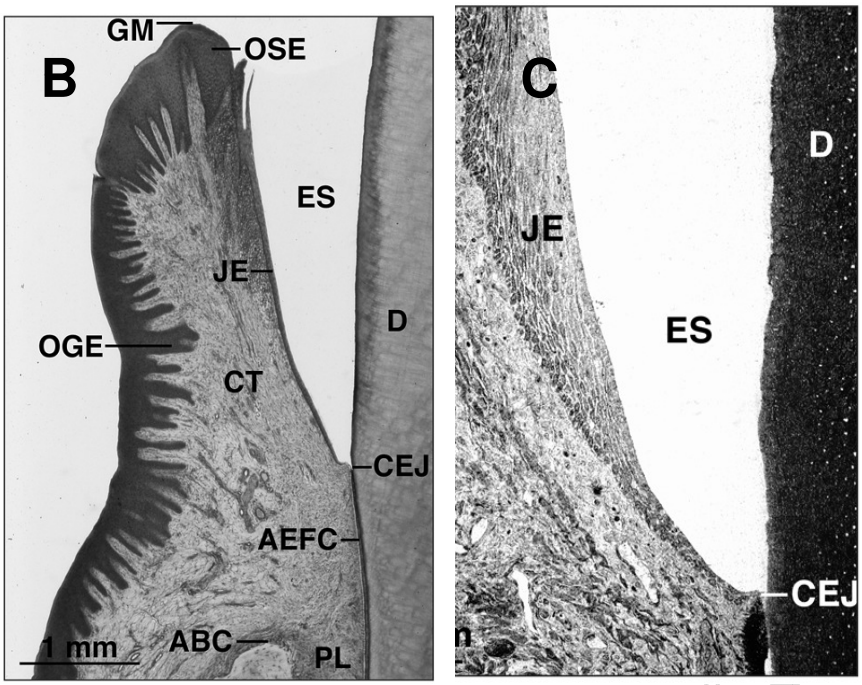
Only time and the imagination of dedicated bench top scientists collaborating freely with industry and astute clinicians can form the kind of synergy that delivers scientific progress and the "consilience of intellect" that Harvard's E.O.Wilson (1998) has implored us to seek, create and nurture. So, for example, when epidemiologists find more periodontal attachment loss in minority adolescents than is normally expected (Cappelli, 1994), a closer look at periodontal effects of orthodontic therapy needs to be initiated. When the New England Journal of Medicine noted that oral infection causes endothelial damage in coronary arteries (Tonetti, 2008) orthodontists responded (MacLaine, 2010). When doctors relate the death of a full term baby to periodontal infection in the mother's mouth (Han, 2010) we must initiate more investigations about the relationship between oral infection and systemic health in pregnant women. This kind of scholastic interaction defines the dentist/scientist as one who is willing to reach beyond the shores of traditional biomechanics and cast a broader net of biological inquiry into the deeper truths of tissue engineering, cellular biology and biochemistry.

In this quest we invite all colleagues, globally, to be one in basic science, and join our consortium of progress, not necessarily without salutary contention. Paraphrasing Goethe, while, talent and insight are nurtured in tranquility, they can only be brought forth by character. And character is forged in a furnace. Interactive scholarship is inherently conflicted, but the wise man finds synthesis and communion in conflict to build common cause. It is with common cause that collaborative progress is achieved.

Above all, Man must always keep faith that the seemingly incomprehensible is merely a truth not yet comprehended. Because, without that faith, and without the willingness to temporarily suspend disbelief for the sake of education, there is no spirit of inquiry. It is that spirit of inquiry into the enigmatic that sustains a spirit of clinical enterprise and specifically the developments in orthodontic stem cell therapy. That is E.O. Wilson's *consilience* indeed.

Figures©

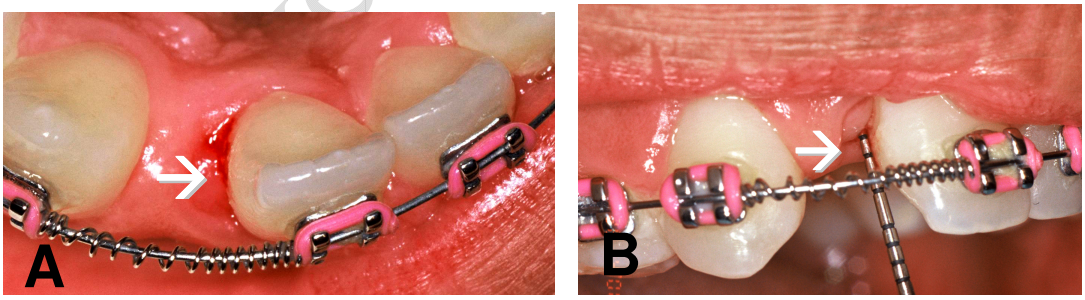




Source: Bosshardt, D.D. Lang, N. P., The junctional epithelium: from health to disease J Dent Res (2005) 84(1):9-20. Used with permission.

Figure 1 Basic Anatomy of the Periodontium.

(A) The periodontium is composed of two parts: (A) The gingival unit, between points 1 and 2 and (B) all periodontal elements apical to point 2 defined as the periodontal attachment apparatus, or informally referred to as "attachment". **(B)** Light microscopic view of young healthy human gingiva. Note: ABC, alveolar bone crest; AEFC, acellular extrinsic fiber cementum; CEJ,, cemento-enamel junction; CT, gingival connective tissue; D, dentin; ES, enamel space; GM, gingival margin; JE, junctional epithelium; OGE, oral gingival epithelium; OSE, oral sulcular epithelium; PL, periodontal ligament. Courtesy of Dr. H.E. Schroeder. **(C)** Back-scattered scanning electron micrograph showing a thinning of the junctional epithelium (JE) as it approaches the cemento-enamel junction (CEJ) in clinically healthy porcine gingiva. CEJ, cemento-enamel junction; CT, gingival connective tissue; D, dentin; ES, enamel space. Courtesy of Dr. A. Nanci



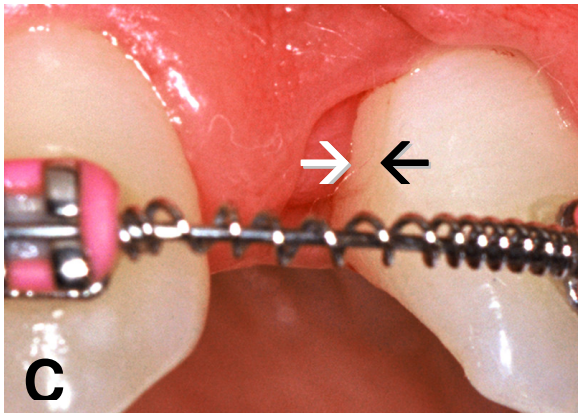
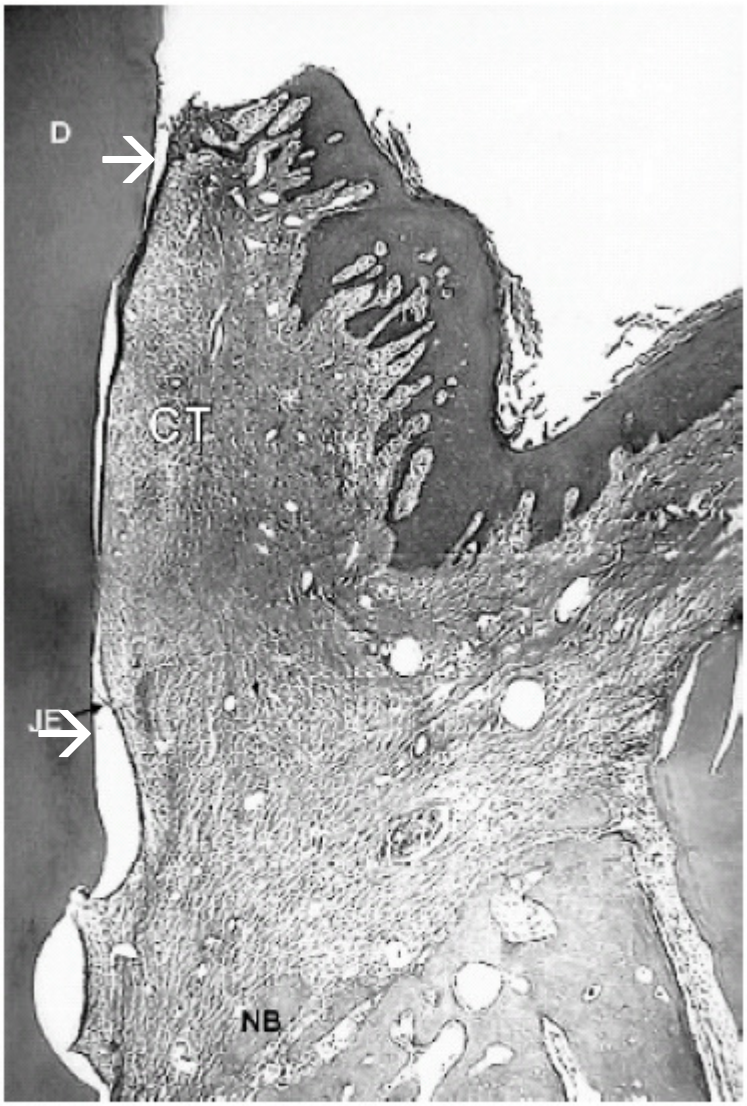


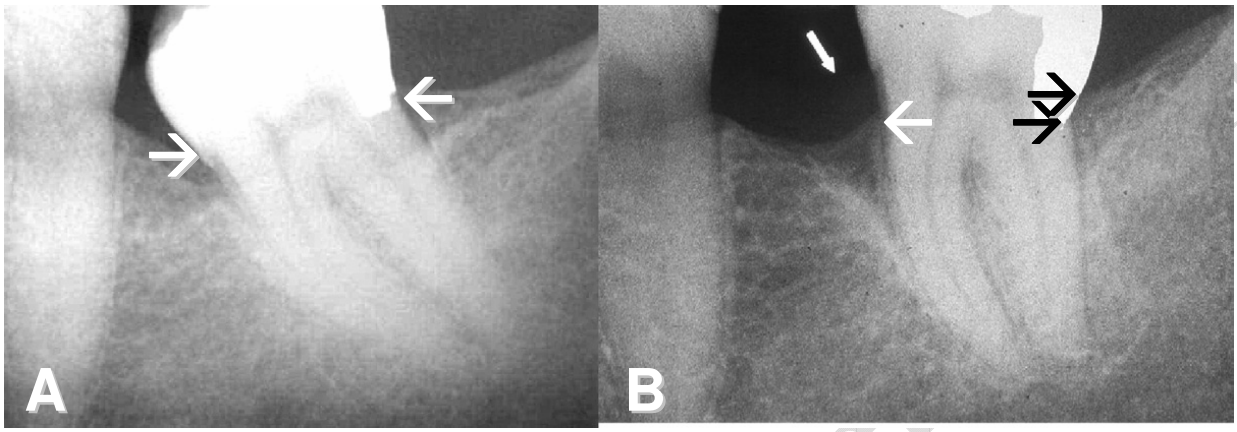
Figure 2 Patient V.M The Red Patch of Atherton. Noted by British orthodontist. J.D. Atherton, [Atherton JD, The gingival response to orthodontic tooth movement Am J Orthod (1970) 58(2): 179-186.] The co-called "Red Patch" is an eversion (prolapse) of the gingival sulcus exposing thin non-keratinized epithelium and subjacent connective tissue of the periodontal ligament. This is a potential source of periodontal attachment loss. If the bacterial biofilm around the recession is benign the everted red patch will mature into marginal gingiva because the marginal gingiva derives from the periodontal ligament and will move with a tooth upon eruption or therapeutic extrusion in health. As this image suggests recession during tooth movement is caused proximately by the negligence of a patient (despite being well informed), not necessarily by tooth movement. Aleo and DeRenzis (1974) showed that destructive endotoxins in dental plaque can inhibit fibroplasia in cell culture. This kind of recession is permanent in the orthodontic patient in the presence of virulent endotoxins and represents the *in vivo* analog of the Aleo and DeRenzis observation. [Aleo JJ, DeRenzis FA, et.al. The presence and biologic activity of cementum-bound endotoxin, J Periodontol (1974), 45(9):672-675.]

(A) From an orthodontist's usual incisal and labial views the everted (prolapsed) sulcus distal to the mesially moving lateral incisor appears curiously benign. But a good clinical scientist *cum* orthodontist looks beyond the obvious and closer inspection (C) reveals a point of vulnerability, or "Disease-Health Nexus", between the white and black arrows. The white arrow marks the coronal extent of a receded periodontal attachment apparatus and the black arrow marks the position it should normally assume in health. This clinical recession anteriorly is obvious; occurring around posterior teeth, it creates infected periodontal pockets and permanent bone loss. These unapparent iatrogenic pockets conceivably would be obscured by hypertrophy and hyperplasia and mark the beginning of progressive periodontitis in the patient's second or third decade. Post-operative periodontal charting and therapy as needed therefore should routinely follow fixed bracket removal. Moreover, the patient should be followed for 1-2 years to ensure orthodontic and periodontal stability. This subtle phenomenon, notable to the vigilant clinician, may be precluded with *in situ* stem cell therapy that employs PAOO™ for alveolus bone augmentation since stem cells tend to repair tissue damage and suppress local destructive immunologic reactions.



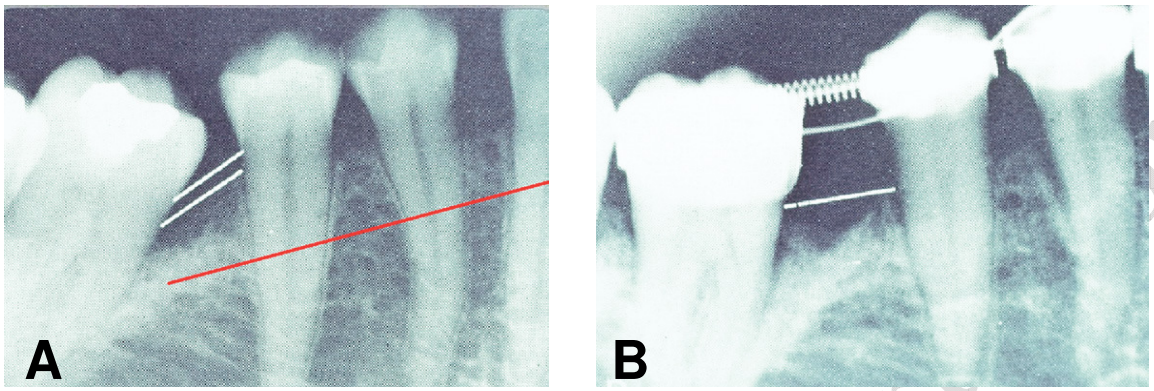
Source: www.scielo.br/scielo.php?script=sci_arttext., .Villaça JH, Novaes AB, Scombatti de Souza SL, et.al. Bioactive Glass Efficacy in the Periodontal Healing of Intrabony Defects in Monkeys, Braz. Dent. J 2005, .16(1) 67-74.

Figure 3 Note the long junctional epithelial attachment (JE) between the two white arrows. The photomicrograph above demonstrates an unusually long junction, attaching the sulcular epithelium and the root surface. The apical extent of the epithelium is labeled JE. This image taken from a specimen to stand in contrast to complete "new attachment" an informal term for complete regeneration of the attachment apparatus on a tooth surface previously denuded by periodontitis. Sometimes clinicians are fooled into believing regeneration has occurred and pockets have disappeared due to gain in regeneration, but actually firm tissue tone and a long JE is only an illusion of regeneration. A long JE is faulty because it breaks down suddenly during orthodontic fixed appliance therapy, often to the chagrin of the hapless orthodontist who believes it may be caused by inferior supportive care or, more naively, the OTM. Such "unzipping" can reveal a true pocket that had pre-existed but was treated with "deep cleaning", a non-scientific term for sub-gingival root planing.. Orthodontists should not be fooled into thinking they are periodontally-safe enough to ignore interactive supportive care from professional team members (including the patient). Tooth movement can indeed exacerbate progressive active attachment loss that is uncontrolled.



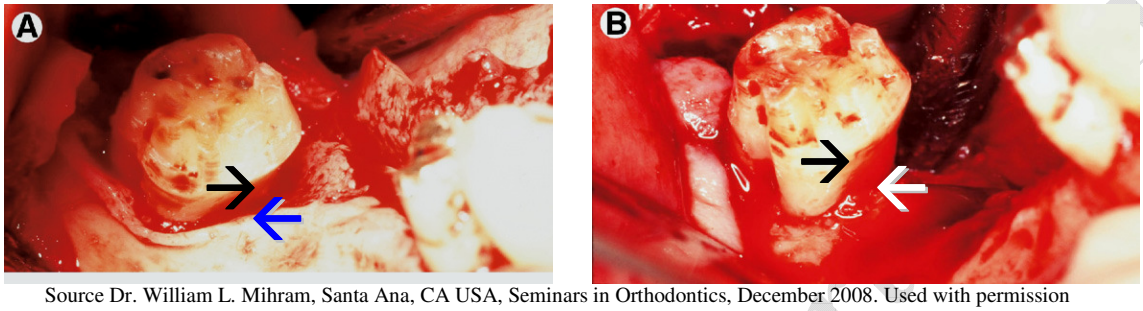
Source: Dr. William L. Mihram, Santa Ana, CA USA in Seminars in Orthodontics, December, 2008. Used with permission.

Figure 4 Note how the attachment level (white arrows) on the mandibular second molar stays at the cemento-enamel junction in health (A) but transforms to a gingival (pseudo) pocket of pathological depth on the distal aspect between the black arrows in (B) when the mesial pocket (white arrow, left) is eliminated by orthodontic uprighting (white arrows right). The small white arrow right indicates the mesial gingival margin. The "bone" level is not sufficiently calcified to register on a periapical radiograph. Full re-calcification of alveolar bone after orthodontic tooth movement takes abut 2-3 years so quick judgments, legal and otherwise, should be made cautiously when referring to "bone loss" around orthodontically moved teeth.



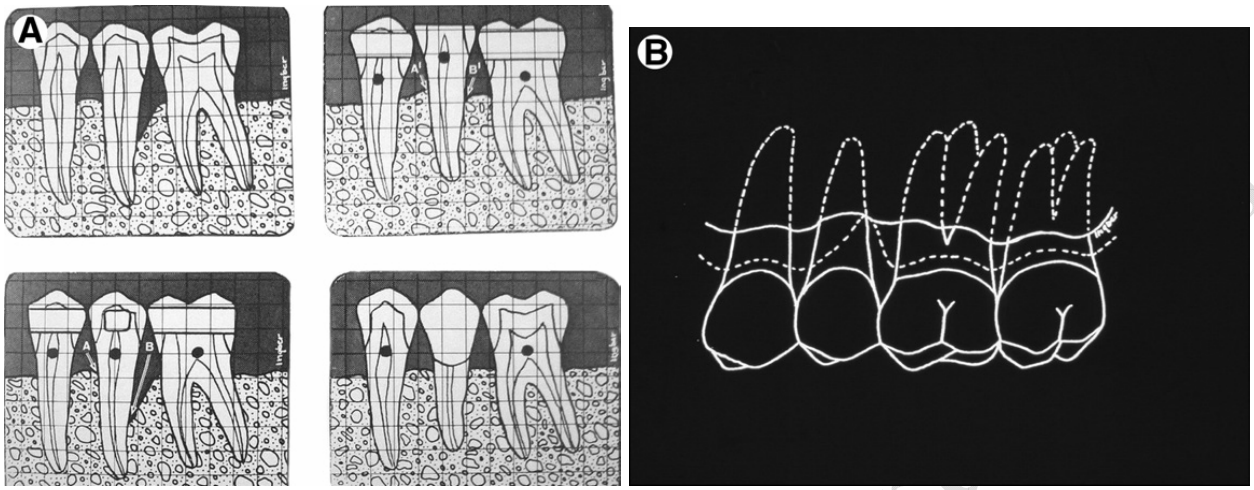
Source Dr. William L. Mihram, Santa Ana, CA USA, Seminars in Orthodontics, December 2008. Used with permission

Figure 5. No treatment better exemplifies the need for so-called "interactive orthodontics" than molar uprighting. If orthodontic therapy is not employed in a conservative treatment plan, then unnecessarily excessive amounts of bone must be removed during periodontal osseous surgery. Bone coronal to the red line in (A) represents the amount of bone that is removed by standard osseous resection when no orthodontic treatment is used to upright the molar. The coronal and apical white lines in (A) and (B) represent the cemento-enamel junction and alveolar osseous crest. The dramatic alteration of alveolar bone phenotype in this iconic representation shows how simple orthodontic aligning and leveling can eliminate the need for osseous surgery. This forms the conceptual basis for alveolar phenotype modification by SAD, PAOO, hMSC placement or viable cell allograft surgical manipulation in the future.



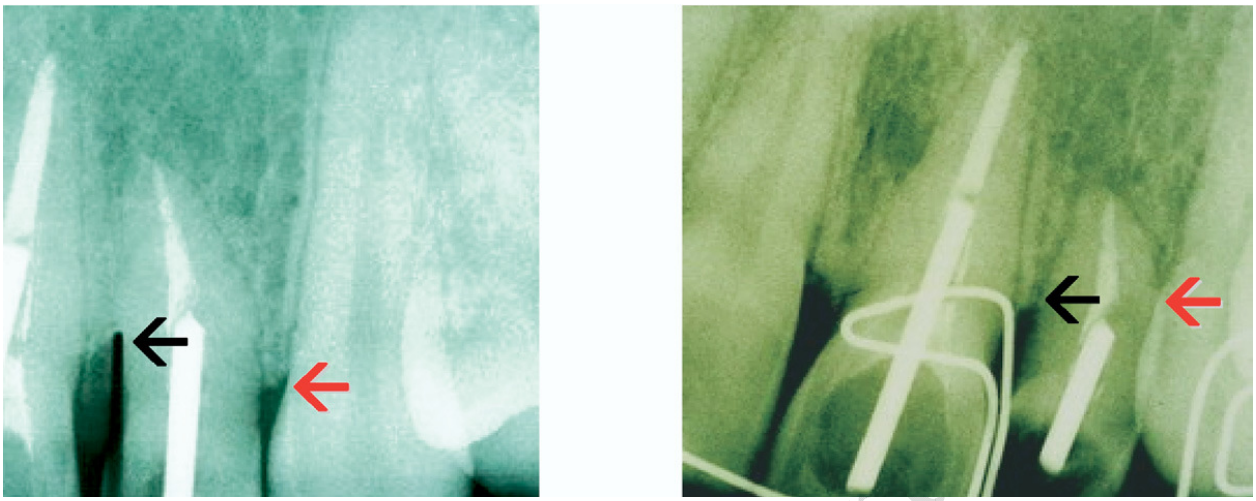
Source Dr. William L. Mihram, Santa Ana, CA USA, Seminars in Orthodontics, December 2008. Used with permission

Figure 6 This image is the clinical correlate of the radiographs in Figure 5. Note how the mesial aspect (black arrow B) of the molar is now clinically available for preparation, instead of hidden by bone (black arrow A). Caries on the mesial surface are often subgingival, even apical to the alveolar crest (blue arrow B) which makes restoration impossible without facilitative (pre-restorative) orthodontic therapy. The surgical flap was replaced at the white arrow in B.



Source Dr. William L. Mihram, Santa Ana, CA USA, Seminars in Orthodontics, December 2008. Used with permission

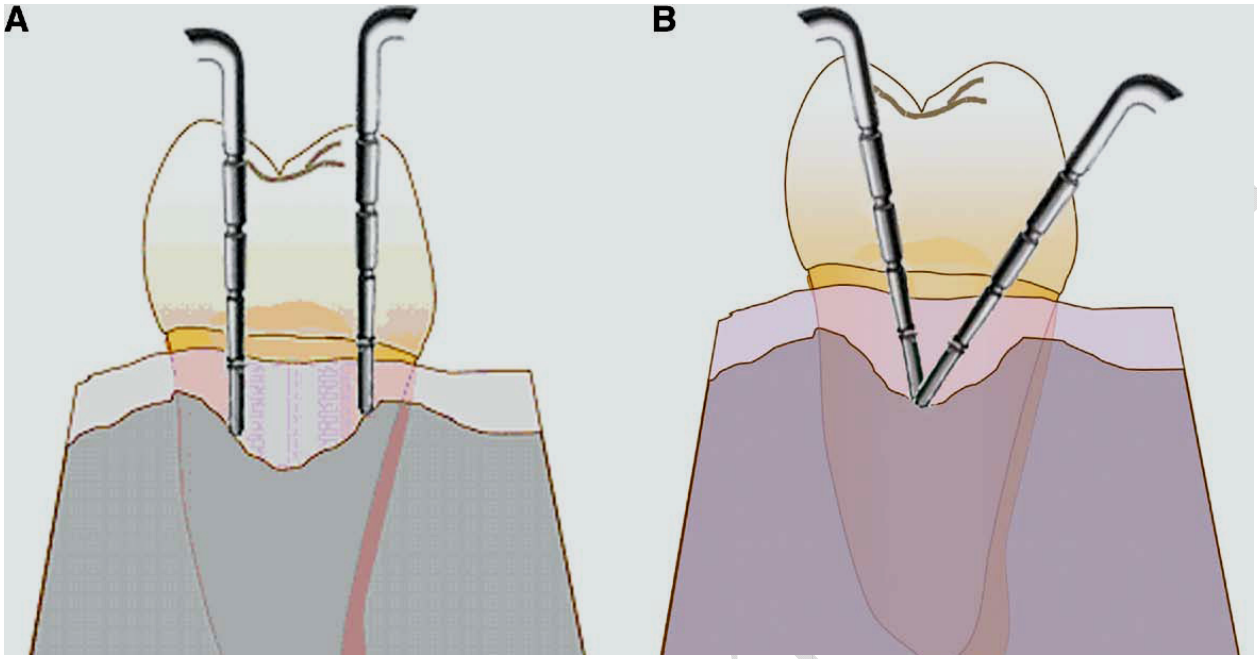
Figure 7 Illustrations from Ingber's definitive articles (1974) illustrate the relationship between the alveolar crest attached to the root in a one-walled infrabony defect A (vertical or angular bone loss) and the effect of facilitative extrusion (forced eruption). The interdisciplinary synergy between the periodontal and orthodontic specialties has made this low morbidity treatment feasible. (B) The vertical (or angular) bony defect that forms the periodontal pocket on the schematic second bicuspid must be surgically recontoured (reshaped by removal of healthy bone) if orthodontic forced eruption is not performed. This unnecessary removal of healthy adjacent alveolar bone can be most dramatic. The pathologic alveolar crest topography (architecture or shape) is represented by the dotted line in (B) The surgical bone removal must involve four teeth (solid line) to blend the architecture of the alveolar crest into a physiological shape because any abrupt change in the topography of the alveolar crest can cause coronal gingival "rebound" after surgical apical positioning. When the crestal topography is gently sloping the gingiva stays at its physiological position next to the bone crest and periodontal pockets do not reform. The morbidity of extraction of the second bicuspid or periodontal osseous surgery are much greater than simple orthodontic forced eruption illustrated in (A).



Source Dr. William L. Mihram, Santa Ana, CA USA, Seminars in Orthodontics, December 2008. Used with permission. Mihran ML, Murphy NC, The orthodontist's role in 21st century periodontic-prosthodontic therapy, Semin Orthod 2008;14:272-289.

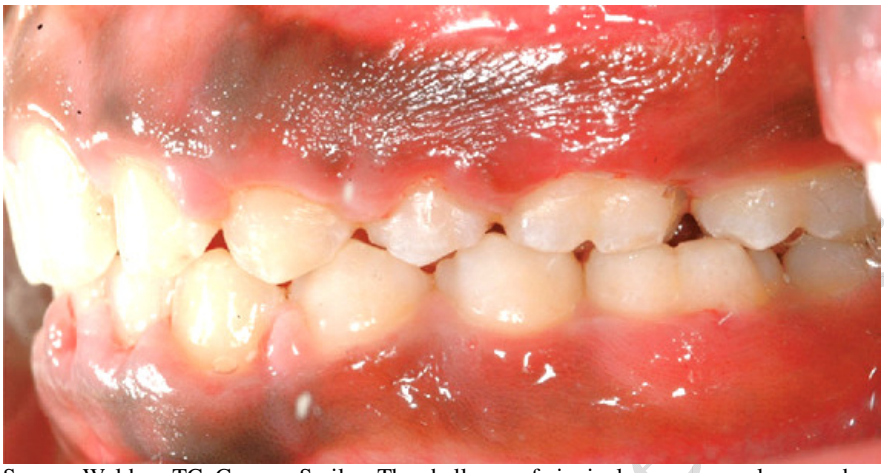
Figure 8 Note how the arrows match in the right image but are asymmetrical in the image on the left, before asymmetrical forced eruption. As the lateral incisor was forcibly erupted with fixed orthodontic appliances the soft tissue fibers were severed periodically to the crest of the alveolar bone between the cuspid and the lateral incisor distal surface.

This allowed full eruption of the attachment apparatus (black arrow left) fixed to the mesial surface of the lateral incisor while the distal root surface was therapeutically moved "out of the bone" to provide symmetrical levels of attachment prior to restoration of the lateral incisor. All this complicated treatment could be obviated by the Holy Grail of attachment gain to the cemento-enamel junction (CEJ) by stem cell reconstructive surgery *in situ* at the lateral incisor mesial surface. Where infrabony periodontal defects present, asymmetrical forced eruption, producing symmetrical crown lengthening, can also be seen as the simple use of partial fiberotomies. A partial fiberotomy on opposite proximal surface keeps bone at consistent level, arrows (red) The reader if encouraged to read about further techniques and complications by consulting the original journal article by Mihram and Murphy in Seminars in Orthodontics, December 2008, where more orthodontic-periodontic treatments and complications are discussed.



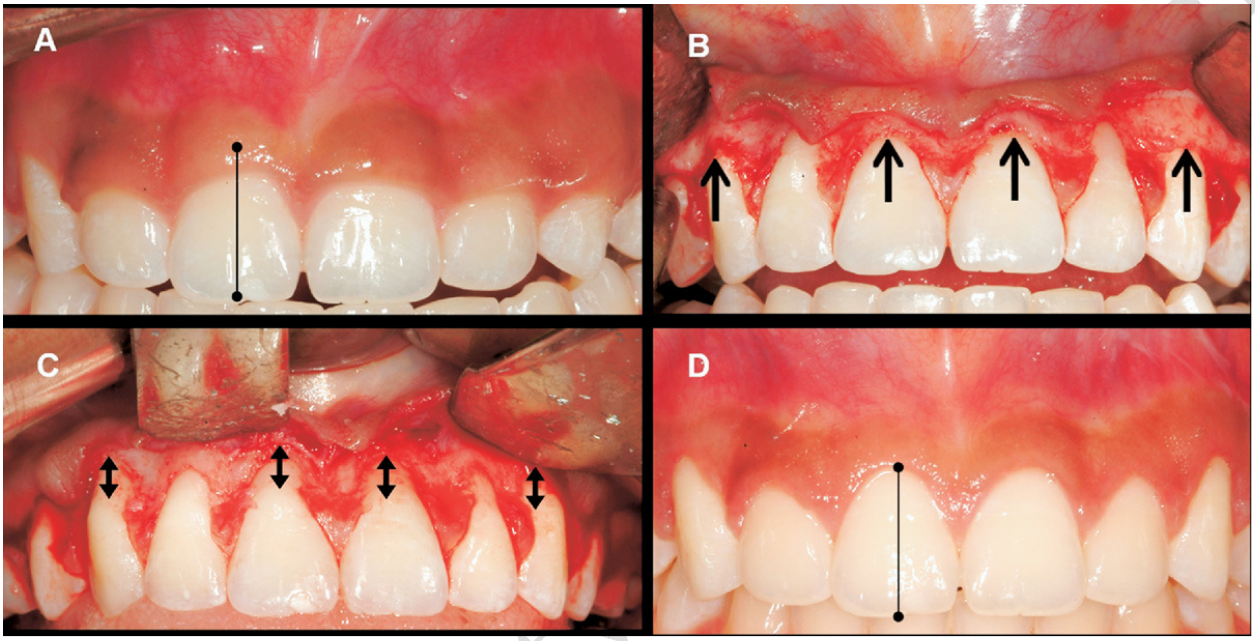
Source: Palomo L, Palomo JM, Bissada NF, Salient periodontal issues for the modern biologic orthodontist, Semin Orthod 2008, 14:229-245. Used with permission.

Figure 9 (A) Probing at arbitrary points such as line angles (image A) may miss defects that are not located in those areas, such as two-walled infrabony defects, commonly referred to as "craters". A common error that misses craters is not advancing the probe far enough inter-proximally, directly apical to the contact point. The periodontal probe is "walked" on the bottom of the sulcus or pocket and angled approximately 20^{o}-30^{o} from the vertical axis (B) between the teeth. This allows the clinician to follow the attachment around the tooth and reach the depth of the pocket that may be more clinically occult and dangerous to future periodontal health.



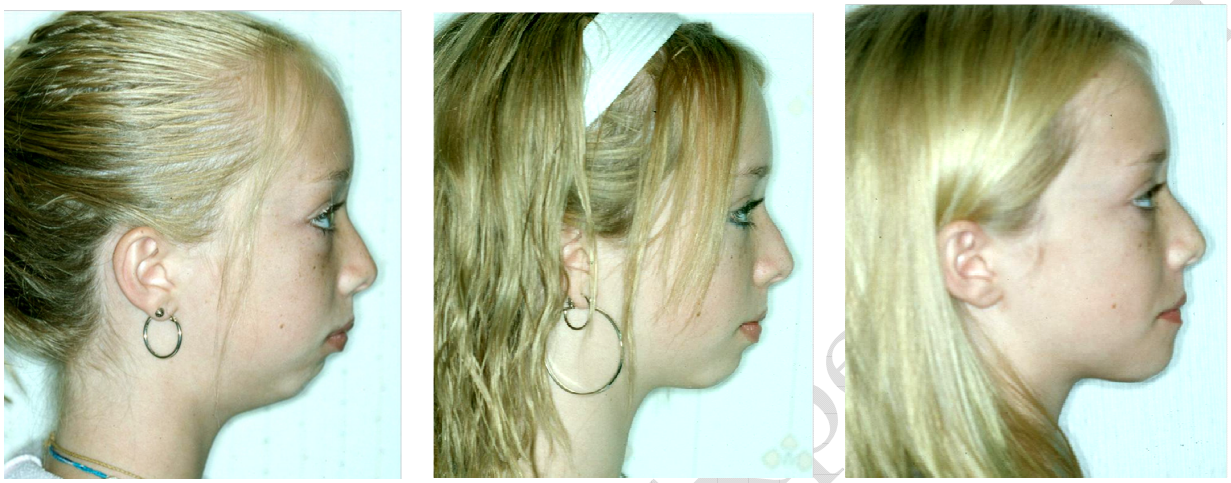
Source: Waldrop TC, Gummy Smiles: The challenge of gingival excess, prevalence and guidelines for clinical management. Semin Orthod (2008)14;260-271. Used with permission

Figure 10 We propose that the case above is not finished according to 21st century standards of periodontics or even orthodontics until the gingival problems are addressed. In the case above one cannot distinguish if gingival enlargement is due to a transient hypertrophy, permanent hyperplasia or altered passive eruption (gingiva and alveolar bone crest). Moreover, The gingival pockets created by this enlargement cannot be distinguished from the incipient attachment loss (even with radiographs) that follows it. Even negligent patients, concerned only about superficial cosmetics can understand that the case above is not finished and will commonly complain about "showing too much gum". With sophisticated 21st century patients making such observations it is necessary for the modern orthodontist to treat with a team of supporting professionals during mechanotherapy. That is the essence of multidisciplinary care and "interactive orthodontics" both administratively and intellectually. The case is "finished" when the gingival margin approximates the CEJ, the patient is fully informed of all treatment options and both the orthodontist and patient has signed an informed consent to interactive or collaborative supportive therapy.



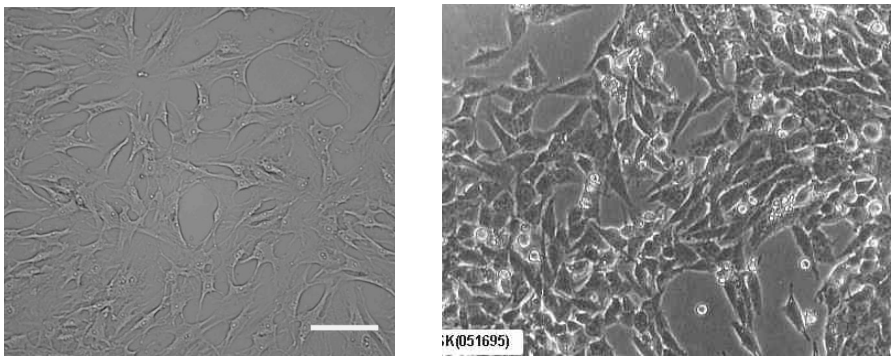
Source: Waldrop TC, Gummy Smiles: The challenge of gingival excess, prevalence and guidelines for clinical management. Semin Orthod (2008)14:260-271. Used with permission

Figure 11 This illustrates the proper method of reestablishing a physiologic biological width and reducing gingival enlargement that occurs in orthodontic cases that use fixed appliance therapy. This precise tissue manipulation and management of underlying bone tissue cannot be achieved with a laser.

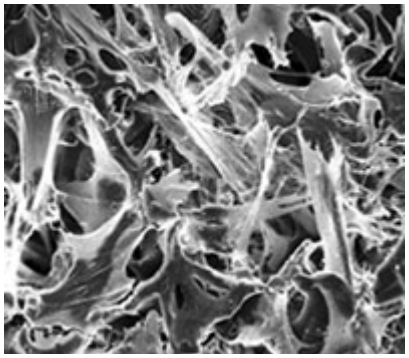


Source: Images compliments of the surgeon, Professor M. Thomas Wilcko, Case Western Reserve University, Eire, PA USA Used with permission

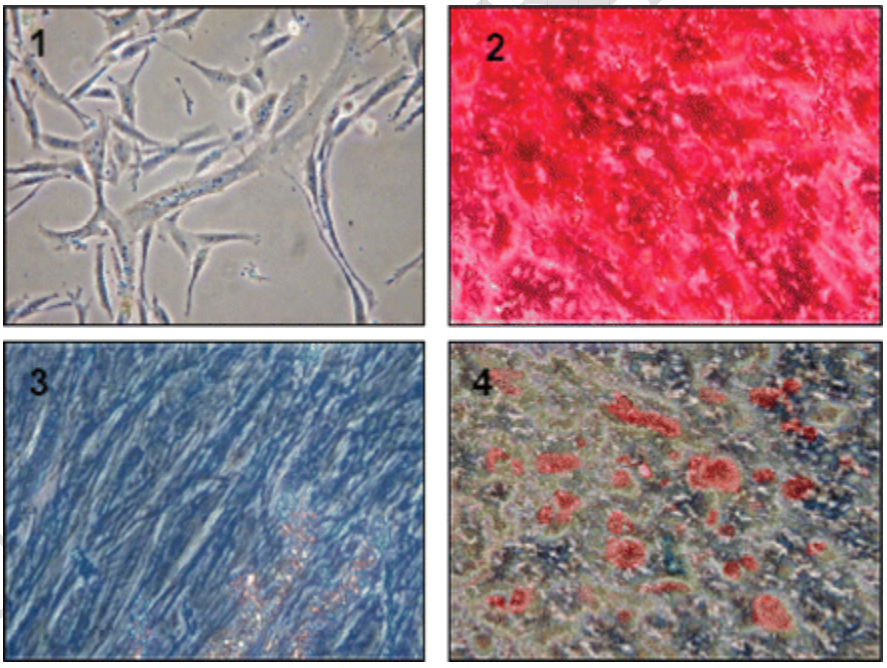
Figure 12 Patient M.K. demonstrates progressive improvement in engineered facial growth. Orthognathic surgery was rejected as an alternative for this case of mandibular retrognathism. Although it is doubtful that the mandibular corpus has been altered, tissue engineering changed the form of the alveolus bone *per se* and the subjacent basal bone inferior to Pogonion. These out-patient surgeries provided not only dental alignment but also satisfactory facial form alternation. The patient was treated with two PAOO surgeries sequentially over a total treatment time of 18 months. The surgery did not involve hospitalization, general anesthesia, or orthognathic techniques; they were entirely periodontal, under IV sedation, as an out-patient.



(Left) undifferentiated mesenchymal stem cells in culture. (Right) culture of rat osteoblasts Note the physical form.
Source: Ryan JM, Barry FP, Murphy JM, Mahon BP J Inflam (2005) 2:8-18

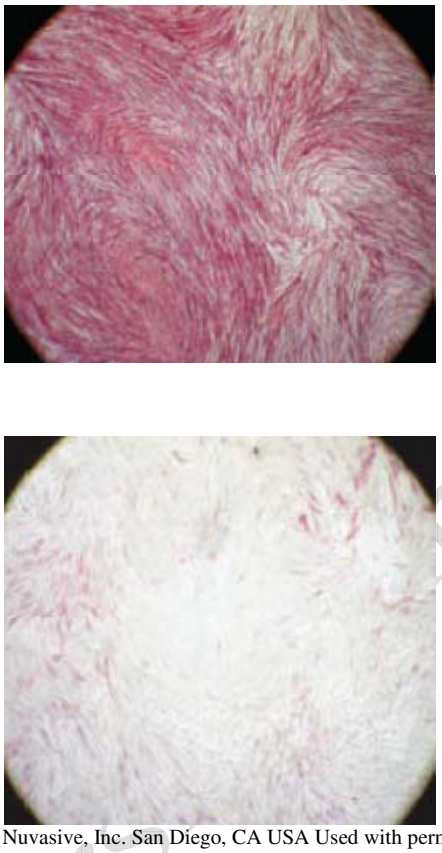


Three dimensional culture of human osteoblasts on novel polystyrene scaffold Source: Nelson B, Thinking in three dimensions, Nature Reports Stem Cells (2007) published online.. Courtesy of Stefan Przyborski, Chief Scientific Officer, ReInnervate Limited



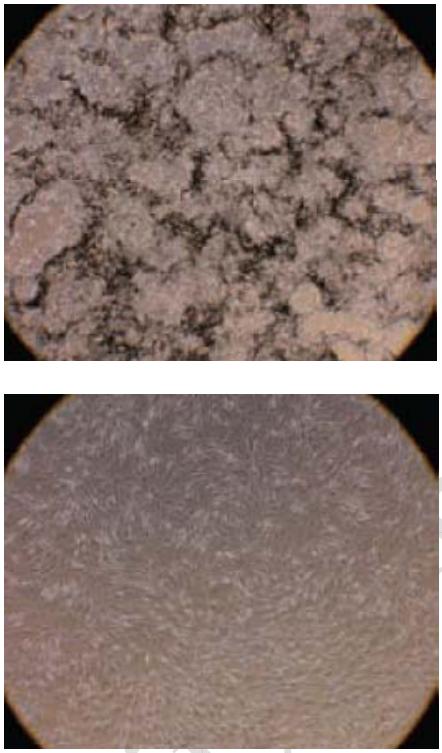
Source: http://bjr.birjournals.org/cgi/content-nw/full/80/Special_Issue_1/S49/F1

Figure 13 The enumerated set of histological specimens, at the lower half of the page, shows a comparison of developmental end fates created by *in vitro* differentiation of human mesenchymal stem cells (hMSC). Cultured hMSCs can enter different cell lineages: (1) an undifferentiated MSC culture as control, (2) osteogenic hMSC, (3) chondrogenic hMSC and (4) adipogenic hMSC lineages. The tissue fate of the stem cells depends on the interaction of local environmental elements such as the interaction of growth factors with mechanical stimuli. However, the exact biochemical mechanisms and the pathways of architectural transcription factors have not yet been clearly defined.



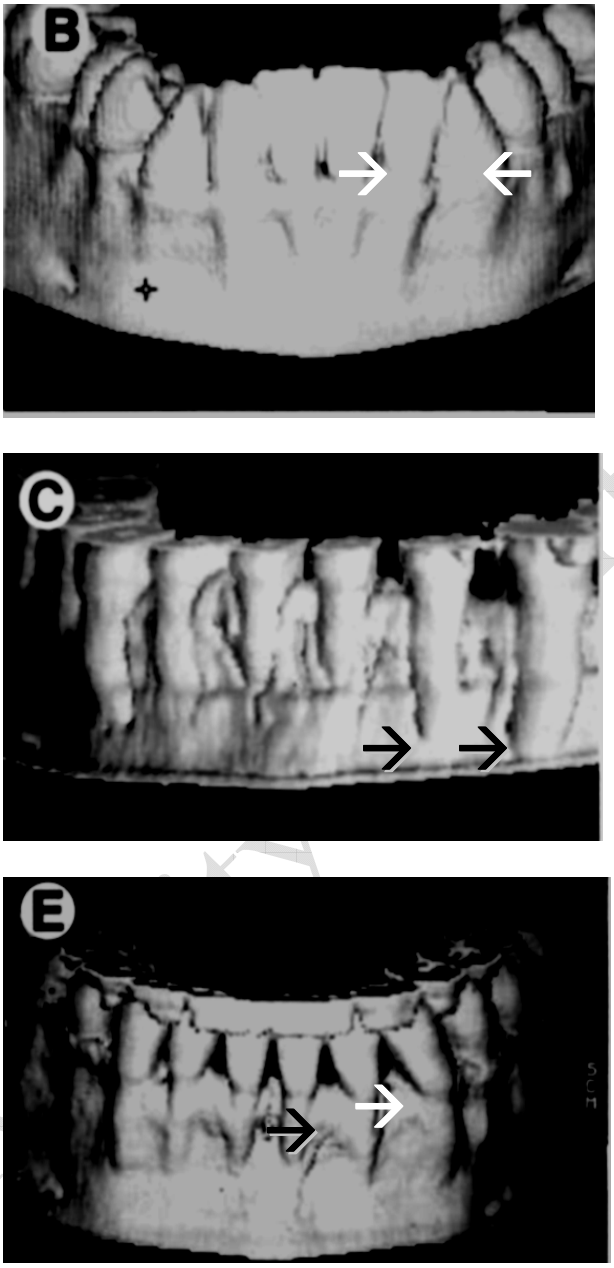
Source: Nuvasive, Inc. San Diego, CA USA Used with permission

Figure 14. Alkaline phosphatase staining is used to demonstrate osteogenesis. Top: Microscopic image of cells positive for osteogenesis. Bottom: Microscopic image of control cells negative for osteogenesis.



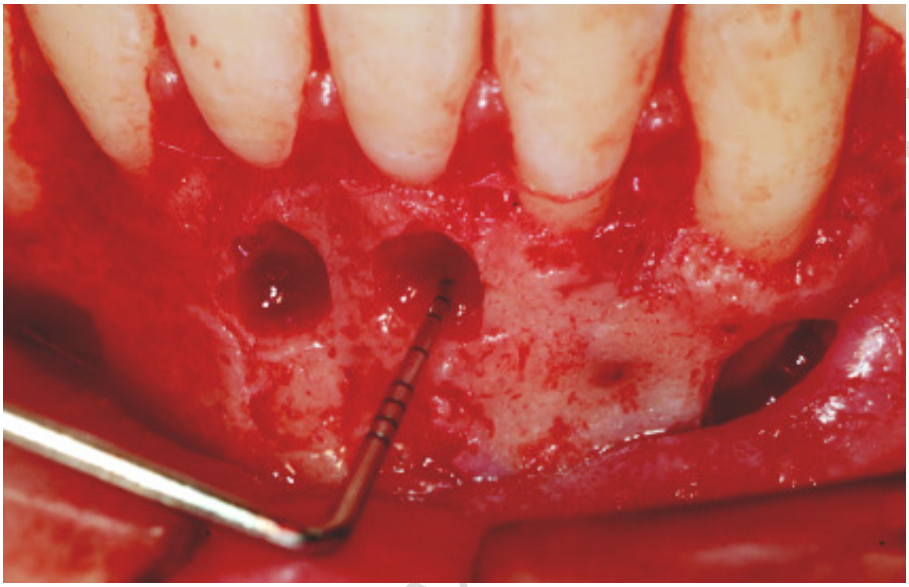
Source: Nuvasive, Inc., San Diego, CA USA Used with permission

Figure 15. von Kossa staining is also used to demonstrate osteogenesis. Top: Microscopic image of cells positive for osteogenesis. Bottom: Microscopic image of control cells negative for osteogenesis.



. Source: Fuhrman, RAW, .Seminars in Orthodontics, 2002, Elsevier. Used with permission.

Figure 16 (B) the alveolar crest is at the CEJ (white arrows). (C) After orthodontic treatment, shows what appears as dehiscence of the labial alveolar bone, where the alveolar crest seems to have retreated to the apex of each anterior tooth (white arrows) and moved "off the alveolar housing". ***Note: This is an illusion***. In (C) the bone is simply less calcified due to the regional acceleratory phenomenon (RAP) of Frost and Jee. (E) three year into retention the alveolar bone on the patients left cuspid has returned to the CEJ and the alveolar bone on the lateral incisor has calcified coronally. Only the central incisors show evidence of permanent bony dehiscence, the limit of individual phenotypic plasticity. Thus, evaluation of final alveolar crest position after orthodontic therapy cannot be made prior to the achievement of "steady state" equilibrium in bone, 3 or more years after debonding.



Source: Professor M. Thomas Wilcko, Case Western Reserve University School of Dental Medicine, Cleveland, Ohio USA 44106 Used with permission

Figure 17 Moving roots into demineralized freeze-dried bone allograft (DFDBA) produces a thick labial mass of cortical and cancellous bone indistinguishable from native architecture with blind evaluation by an oral pathologist. It is proposed that following the same surgical protocol with hMSC allografts will produce identical results, faster, and with fewer surgical complications, side effects, or sequella, e.g. erythema, inflammation, edema. The thesis this image demonstrates is that thicker bone makes orthodontic clinical outcomes faster, safer and more stable by altering genetic expression to re-define the limits of the phenotypic spectrum.

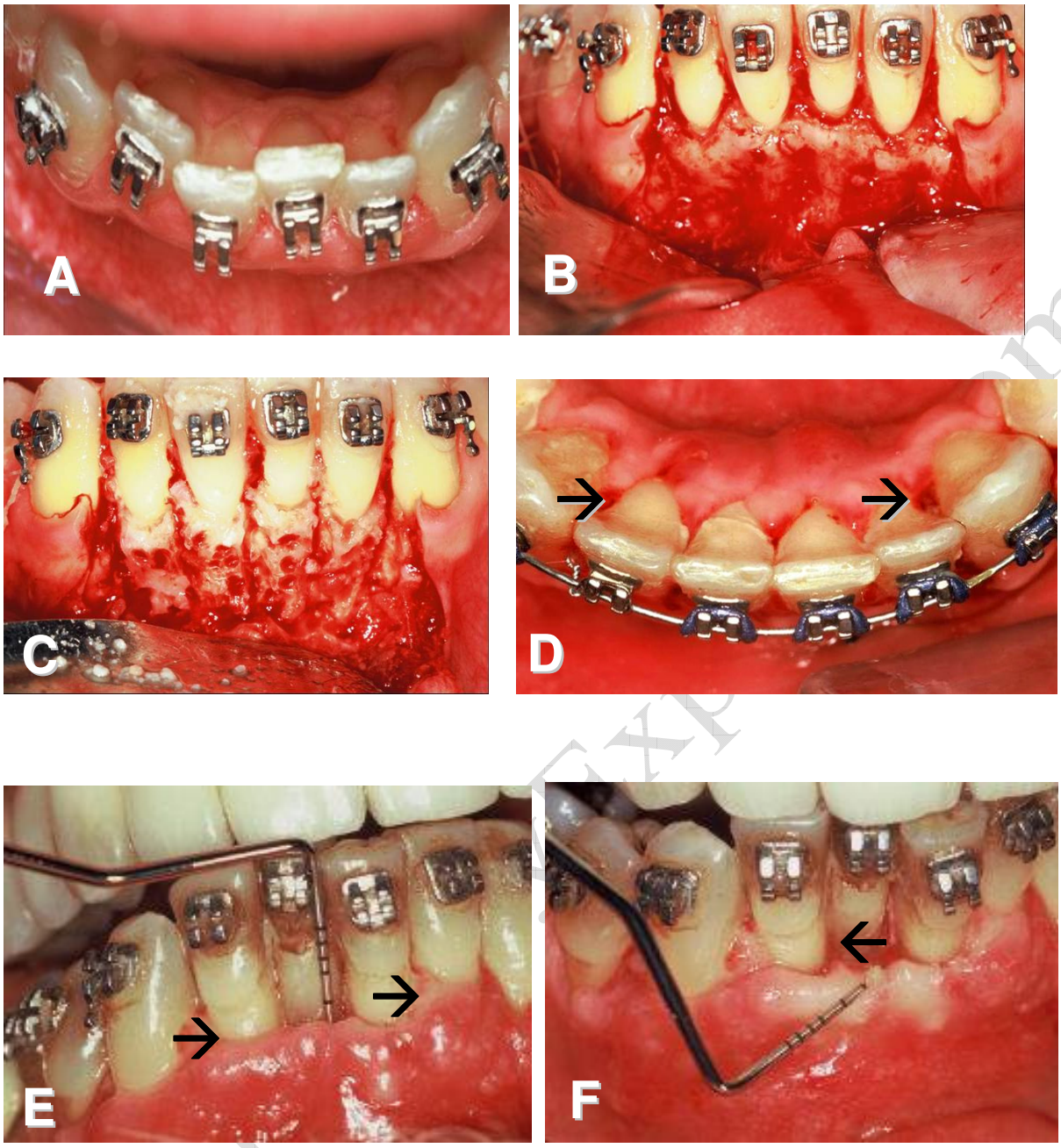


Figure 18 (A-D) Patient A.A.,(A-D) a 22 year old male, presented with mandibular incisor crowding as defined by the OldThink "arch length deficiency (ALD)" * The patient elected SAD (images B and C). He was appointed for post-operative inspection 10 days later. At the post operative visit, the patient reported that his teeth were "perfectly straight in 4 days". Stem cell grafting with hMSC would have improved this outcome by reducing the amount of marginal inflammation seen in post-operative image D (arrow).

Figure 18 (E-F) Patient E.O. The absence of an inflamed surgical margin in image (E) is characteristic of the rapid healing seen in the stem cell graft patient (Patient E.O) and the rapid regeneration (arrow) in image (F) More rapid healing with less inflammation is characteristic of hMSC grafts and the reasons may be related to the secretion of cytokines an other factors that suppress a local immune response, "rejection" responses, and graft vs. host disease.

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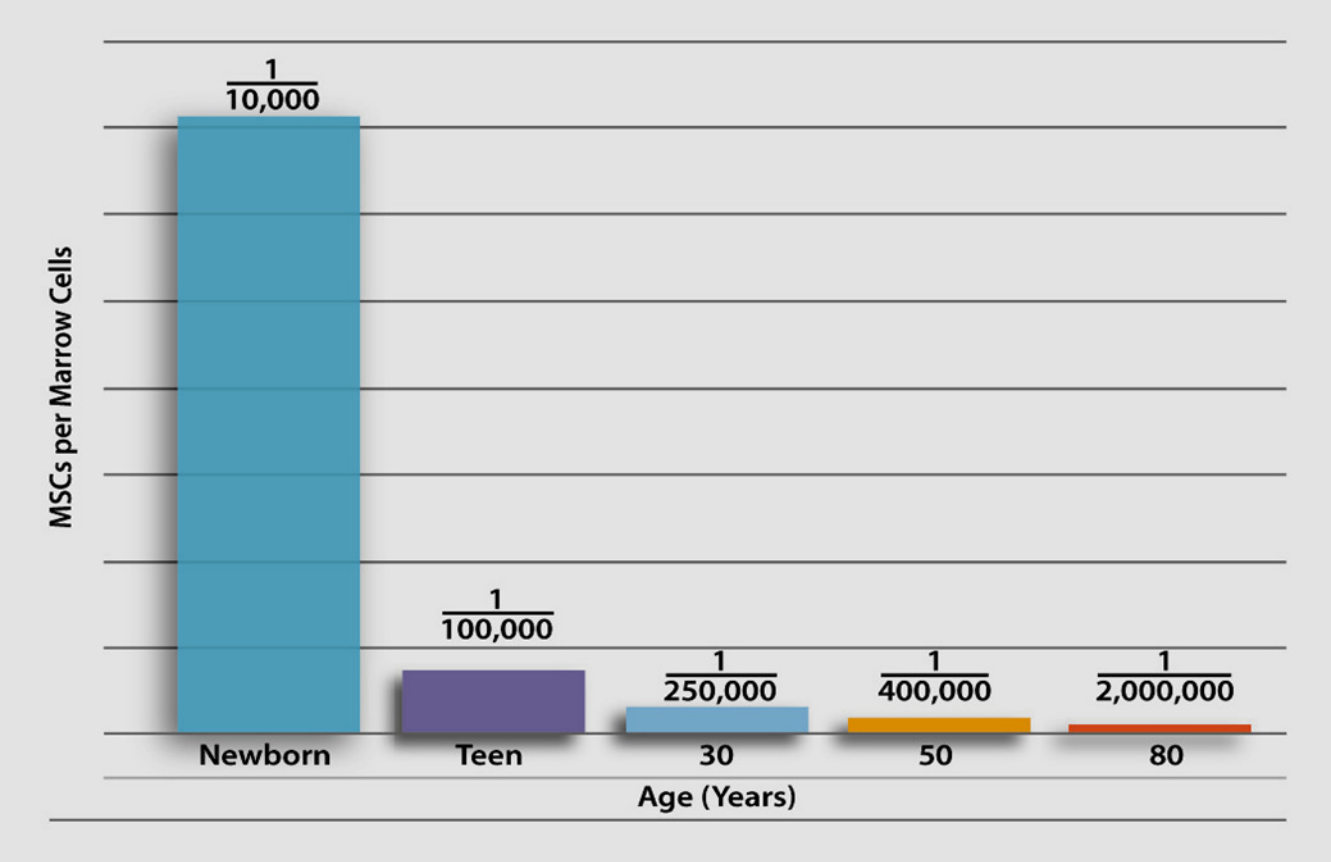
* This text demonstrates that available arch length should not be defined by the dentition, but rather as the labial-most dimension of the alveolus into which teeth may be moved or bone augmented.



Source: Caplan, A 1994.Images compliments of Orthofix, Inc. San Diego, CA USA Used with permission.

Figure 19 The concentration of stem cells diminishes rapidly with age.

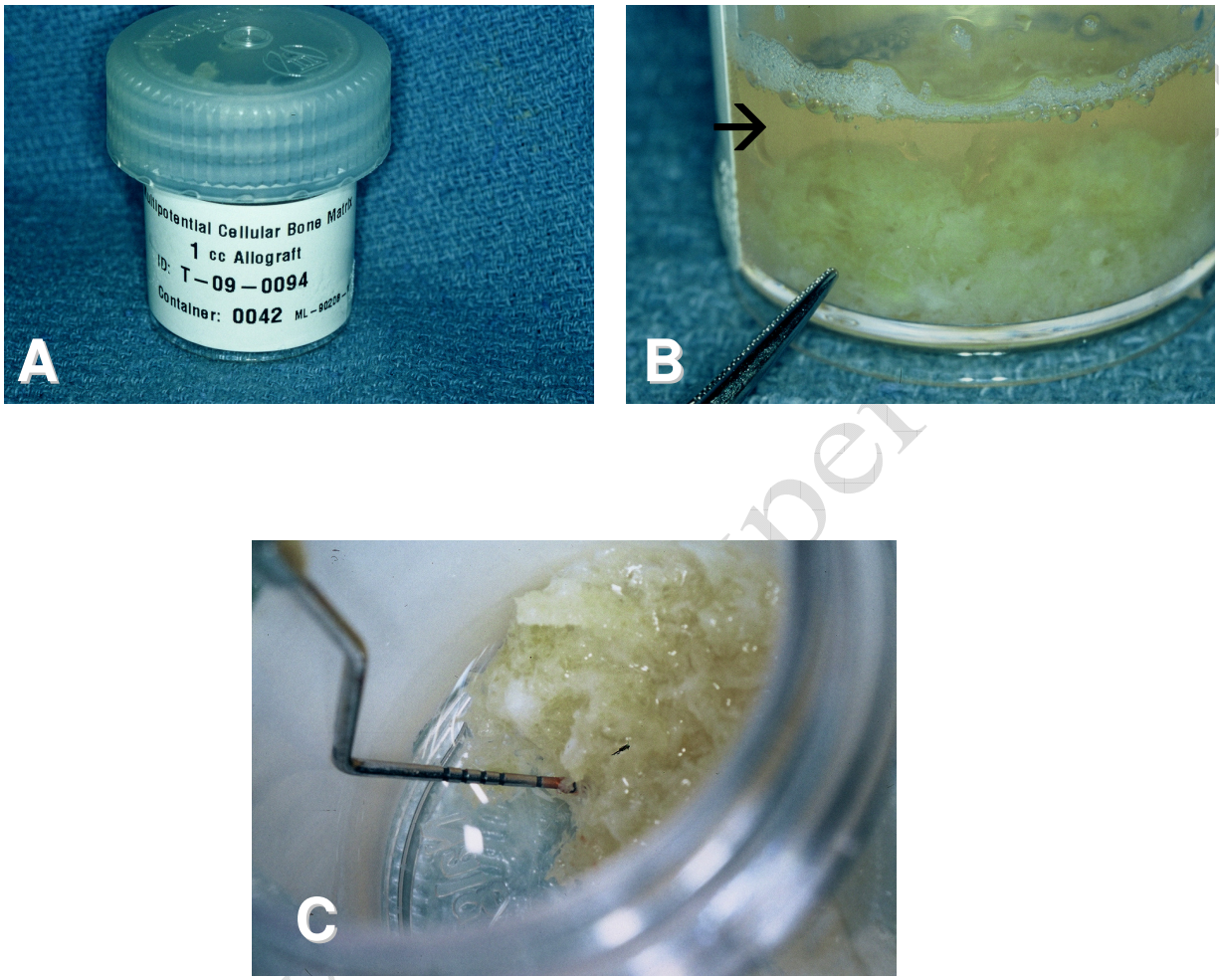
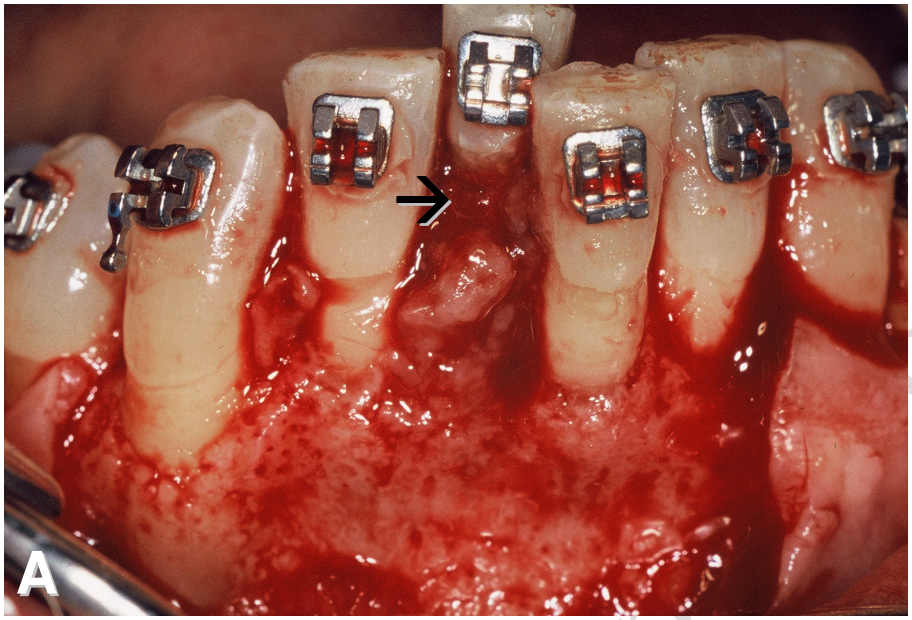


Figure 20 (A) The package containing mesenchymal stem cell (MSC) allograft (B) Supernatant (black arrow) is a minimal essential medium (MEM) and cryopreservative which maintains viability of living stem cells (viable cells) in the allograft after chairside thawing. The allograft (at the tip of the clinical instrument) has settled to the bottom of the container. (C) After the supernatant essential medium is poured off, the MSC allograft is soaked in a "bath" of Clinadmycin 150mg/mL for 15 seconds, and then the graft is ready for immediate placement on the recipient bed of decorticated alveolus.



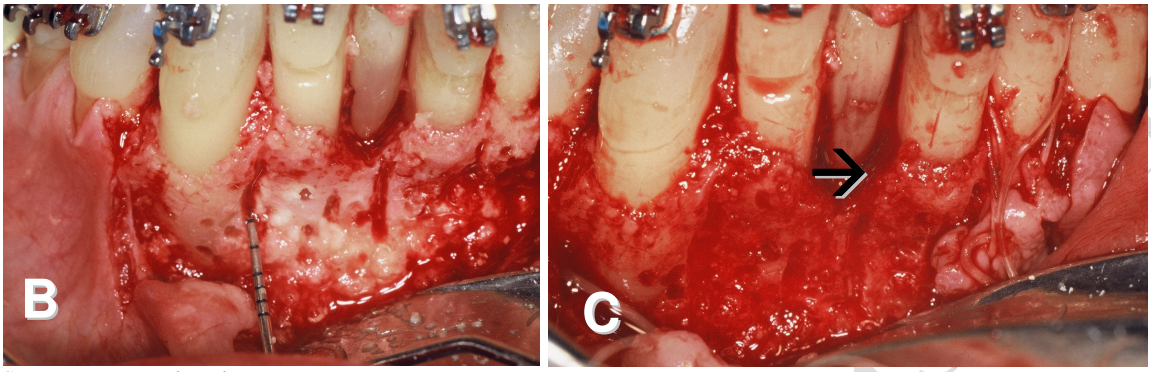
Source: Dr. Neal C. Murphy, CWRU, UCLA www.UniversityExperts.com

Figure 21 (Patient E.O.) Before the alveolar bone is decorticated to receive the hMSC allograft all granulation tissue (arrow) and root accretions should be removed with standard periodontal debridement techniques and root planing. This case demonstrates that the so-called "compromised periodontium" is no less amenable to orthodontic tooth movement (OTM), SAD, PAOO or hMSC grafts than a healthy dentition, given that all infective elements on the roots are eliminated. OTM can be achieved for actively infected patients, presenting so-called "hot lesions" if comprehensive root debridement is done well, just prior to decortication and stem cell placement.

Protracted initial periodontal therapy, such as scaling and root planing (S/RP), informally known as "deep cleaning" produces a "cold lesion" that is less receptive to regeneration or phenotype change. Ironical and heretical as it may seem, 6-8 weeks of S/RP can actually inhibit regenerative potential by eliminating high concentrations of growth factors that accompany local inflammation. It is helpful to note that the patient is in a "healthy state", well prepared for regeneration or tissue engineering, merely seconds after the last root accretion is removed. Meanwhile, the soft issue is the flap is loaded with growth factors ready to aid the healing wound.

Also, a healthy dentition with less than normal support is no more vulnerable to premature tooth loss than a fully supported dentition. *Bone loss* must never be conflated with its cause, *active disease*. They are two separate intellectual entities that may or may not be related. Just as a limping weak leg is not poliomyelitis but rather the result of the infection, so bone loss *per se* is not the disease but rather the result of the infection.

In this particular case, once the periodontal "hot" lesion on tooth #25 is debrided and grafted, orthodontic therapy begins immediately. Although the periodontal literature preaches against graft "micro-movement" the principles of regeneration are not necessarily compromised by induced internal bone strain, when new phenotypes are regenerated through root movement, e.g. PAOO. So infrabony regeneration and alveolar phenotype alteration can occur concomitantly. Orthodontists commonly make a mistake by failing to draw a distinction between stable (inactive) attachment loss and the actual infectious disease process itself. Interactive orthodontists solve this problem which insular orthodontists must live with to their detriment.



Source:: www.UniversityExperts.com

Figure 21 (B) Demonstrates how punctate and linear decortication frees endogenous mesenchymal cells with about 2-3 mm penetration into the spongiosa (see probe). Stem cells from the marrow of the patient thrive in a field of copious bleeding so the virtue of traditional surgical homeostasis is questionable in some cases. (C) Shows that active bleeding should be evident before the MSC allograft is placed on the recipient bed of decorticated labial alveolar bone. Note debrided and decorticated infrabony pocket on tooth # 25. (arrow) is specifically treated to elicit copious bleeding. This is encouraged where *in situ* stem cell grafts are placed or when a mixture of so-called "viable cell allografts" is used

For the sake of syntactical clarity a technical distinction must be made between this kind of *in situ* stem cell therapy or "viable cell allograft" shown here and intravascular stem cell therapy use in other disciplines. Nonetheless one should not view this procedure as tantamount to traditional DBM or DFDBA regeneration. That common protocol only reestablishes a preexisting phenotype. This "stem cell therapy" engineers a new phenotype, better designed for tooth movement and responding epigenetically to it. The guiding maxims are: "wound healing recapitulates regional ontogeny" and "stressed bone wounds heal differently than bone in steady state equilibrium". Instead of seeking immobilization to preclude what medical orthopedists would call an osteopenic "malunion" we intentionally deliver internal strain gradients to the wound in order to "re-program" or, in more correct biologic terms, "imprint" the hMSC, OPC and osteoblasts in this viable cell allograft.

We propose that clinical phenotype change is achieved at the cytoskeletal level by re-engineering the delivery of novel architectural transcription factors to the nucleus of the stem cell. This cell level engineering is not possible with standard bone grafts such as DBM, DFDBA and manifestly not happening with conventional orthodontic mechanotherapy. (See Murphy, 2006 in reference section or at www.universityexperts.com)

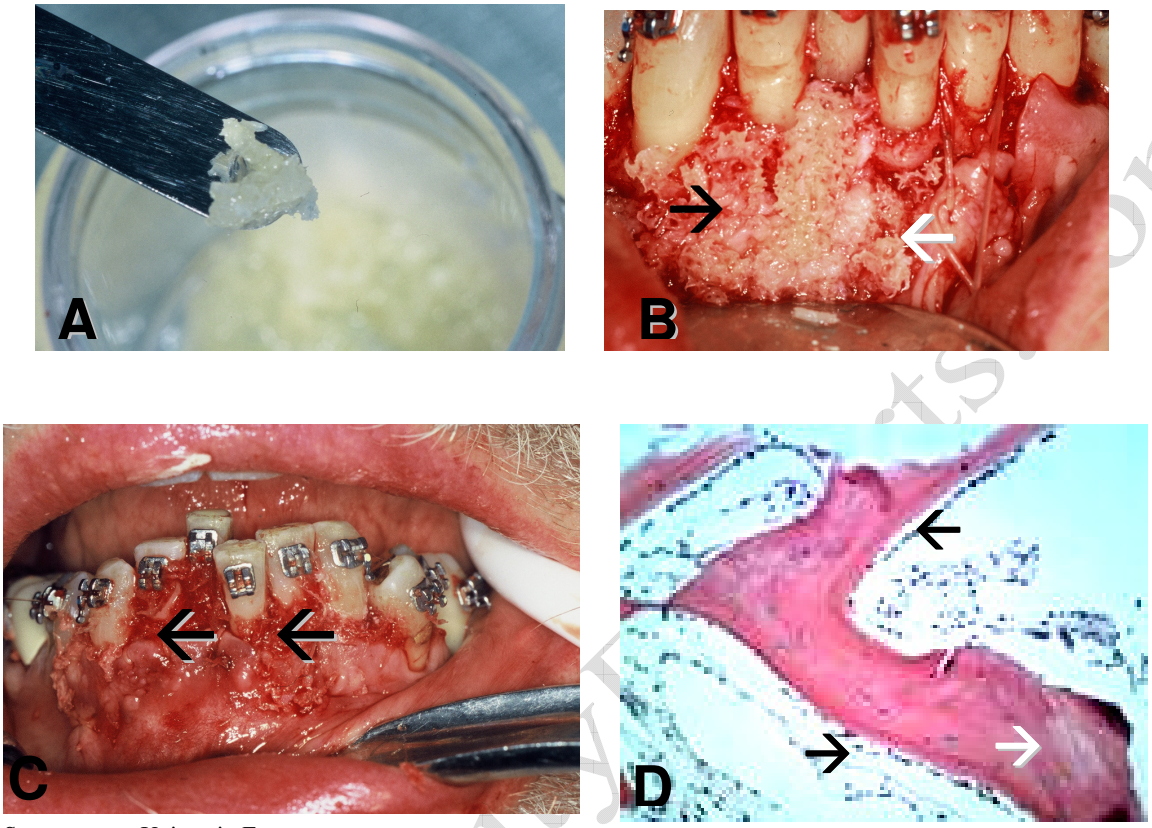


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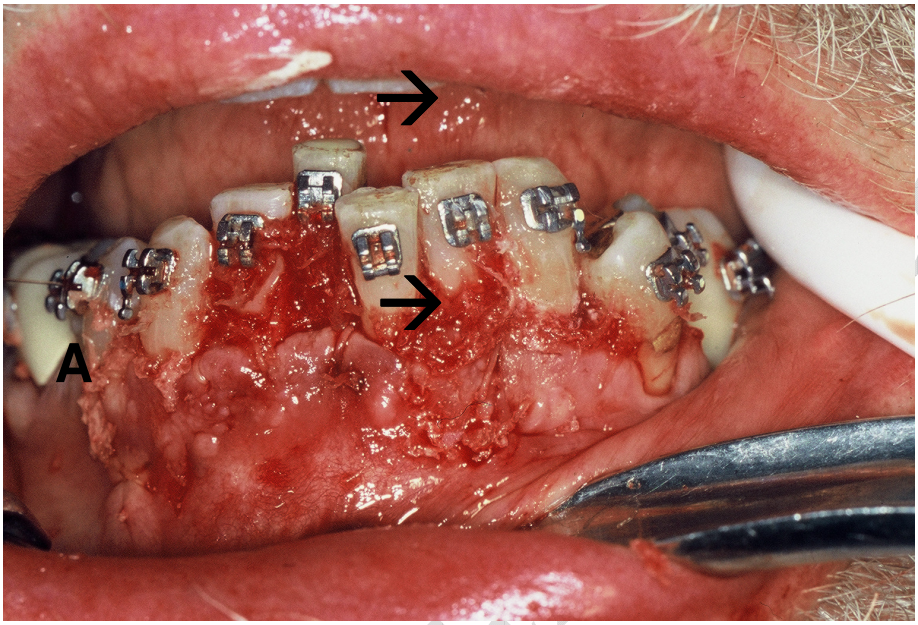
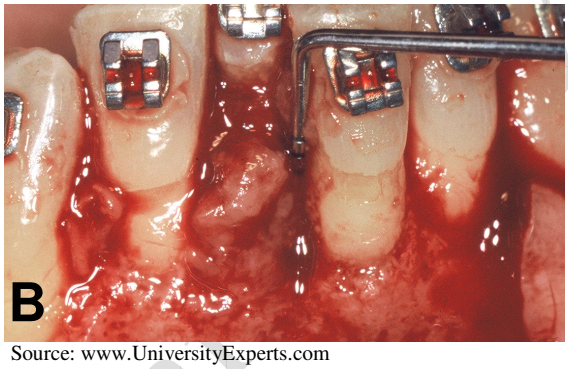
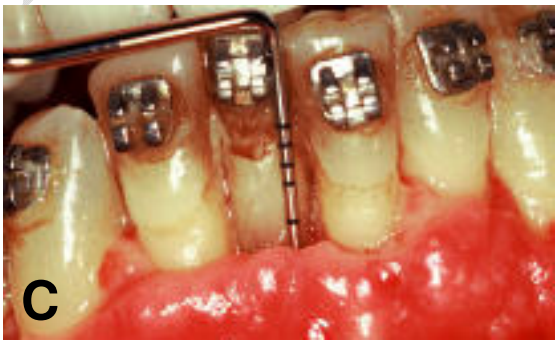
Figure 22 (A) A simple sterile spatula can transport hMSC allografts. (B) The stem cell allograft is molded to the prepared recipient site, (black arrow) and tucked under the loosely sutured mucoperiosteal flap The continuous locking suture (white arrow) is then drawn over the MSC like a purse string.

(C) After suturing has coronally positioned the surgical flap for patient E.O. and held the graft against the decorticated labial alveolar bone, a covering of cyanoacrylate (black arrows) ensures that the flap is immobilized and sutures are secure. The cyanoacrylate, hardened to the sutures and the edge of the flap acts like an artificial "scab" to ensure stability of flap and sutures. It often falls off the graft site when healing is sufficient to hold the graft, usually in 2-5 days. A 0.018" nickel-titanium round archwire was placed immediately after the cyanoacrylate cured so tooth movement could commence immediately into the graft providing therapeutic strain gradients on the decorticated bone and hMSCs. Because wound healing recapitulates regional ontogeny, (Murphy, 2006) it is hypothesized that these physiologic strain gradients, estimated at 500-1,000 microstrain will allow the stem cells to differentiate into daughter cells and osteoblasts, move labially to redefine local phenotype and increase labial alveolar bone mass. This is the logical synthesis of the work of Wilcko, Ferguson et.al. and clinical tissue engineering and the Utah Paradigm, a fundamentally new approach to clinical orthopedics. See Suggested Reading)

(D) Histologic analysis confirms normal healing of bone *de novo.* The movement of tooth #25 labial has genetically reprogrammed a new supporting phenotype that lends a more stable outcome than conventional orthodontic treatment and a better overall quality even when compared to standards of the American Board of Orthodontics (ABO). This is an historical mandate for change.







Source: www.UniversityExperts.com

Figure 23 (A) the sutured flap is secured with a blanket (black arrow) of tissue adhesive (cyanoacrylate) when buccal-lingual primary closure is not possible. (B & C) The gain in attachment documents the efficacy of the techniques.

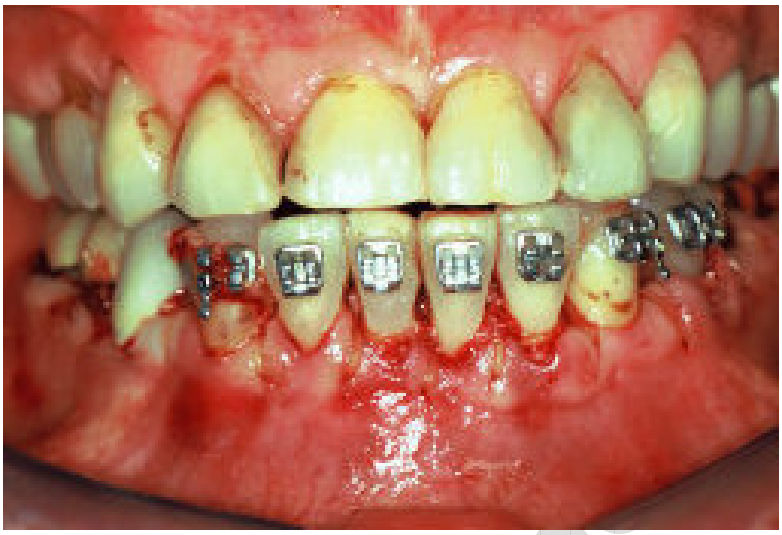
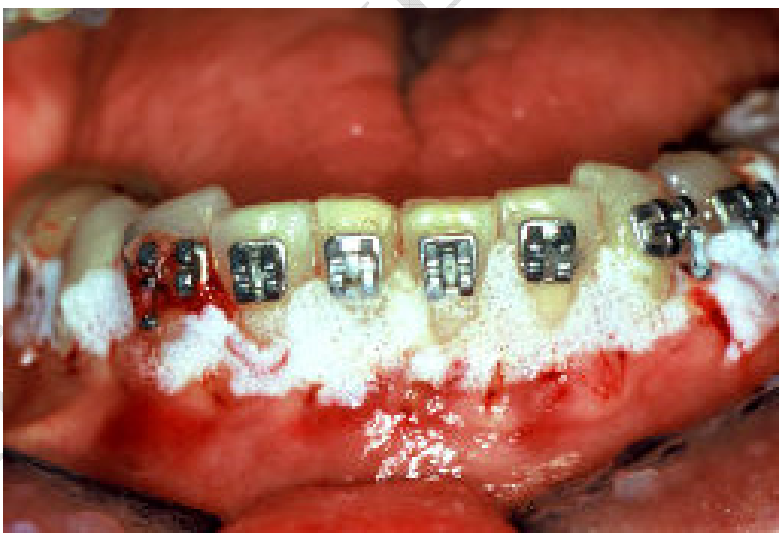
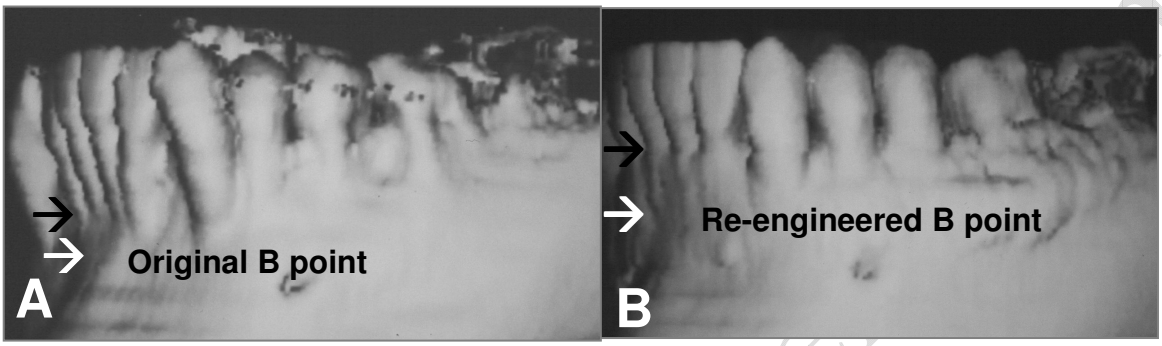
…… … 

Figure 24 A The suture position between teeth #25 and #26 is ideal to protect the growing stem cells. The suture position between #23 and #24 was repositioned prior to a cyanoacrylate ” blanket” placement.

. 

Source: Dr. Neal C. Murphy CWRU, UCLA www.UniversityExperts.com

Figure 24B The final position of sutures and cyanoacrylate blanket prior to patient dismissal. The cyanoacrylate will discolor over the following 2-3 weeks and fall off the teeth, just like a natural scab, when the subjacent tissue matures. In cases like this, which combine periodontal regeneration, whether one treats “hot lesions” or “cold lesions”, is a matter of individual doctor discretion and is derived form practice style and patient preferences. Usually 25%-85% regeneration of infrabony defects is possible as the teeth align and the new alveolus phenotype calcifies to a native architecture. This protocol incorporates 3 objectives that would require separate treatment. By combining all procedures into one, all costs (e.g. financial, biologic) of comprehensive care is reduced significantly.



Source: Professor M. Thomas Wilcko, Case Western Reserve University School of Dental Medicine, Cleveland Ohio USA 44106 Used with permission

Figure 25 In contrast to periodontal regeneration, which merely re-established original form, this figure demonstrates a kind of re-engineering to a novel phenotype. It is still within a spectrum of genotypic potential, but newly designed to contain tooth root position. This exemplifies the validity of Professor Moss's Functional Matrix hypothesis which explains how the roots of the teeth are the "functional matrix" (template) for new alveolus bone.

A new concavity radius forms at B-Point after the PAOO/AOO bone graft is mature as the *Wilcko Curve*. It has morphogenetic significance because it defines the mature labial convexity at B point. In terms of its morphogenesis we postulate that it is defined by the angle of the lower incisors to the mandibular plane at the point of regional ontogenic maturity.

For some time after surgery the hMSC or allograft in this area may actually appear as a convexity due to the bulk of the graft. At this point in time, if orthodontic stress is not applied to the bone and the Frost/Jee Regional Acceleratory Phenomenon (RAP) is not employed through the original convexity, a clinical "bulge," relapses back to the original B-Point curvature as the entire bone graft resorbs. The standard rationalization for such previous failures is "You cannot grow bone on a flat surface". However, using PAOO with a stem cell component, the original clinical bulge and convexity can model to a new phenotype appropriate to the teeth in a treated position. This is why PAOO is so stable. But the treating orthodontist must be patient because the final Wilcko Curve may not fully define itself until 3 years into the retention phase. When it finally does, it serves as both a radiographic landmark for morphogenetic homeostasis and histologically, "steady state" equilibrium for the new labial bone. Note the different radius from B-Point in the images above (white arrows). The linear distance between the white and black arrows demonstrates new attachment apparatus and alveolar bone support that was engineered as appropriate the roots in a more physiologic position. Stem cell allografts can make this surgical engineering safer, faster and better.

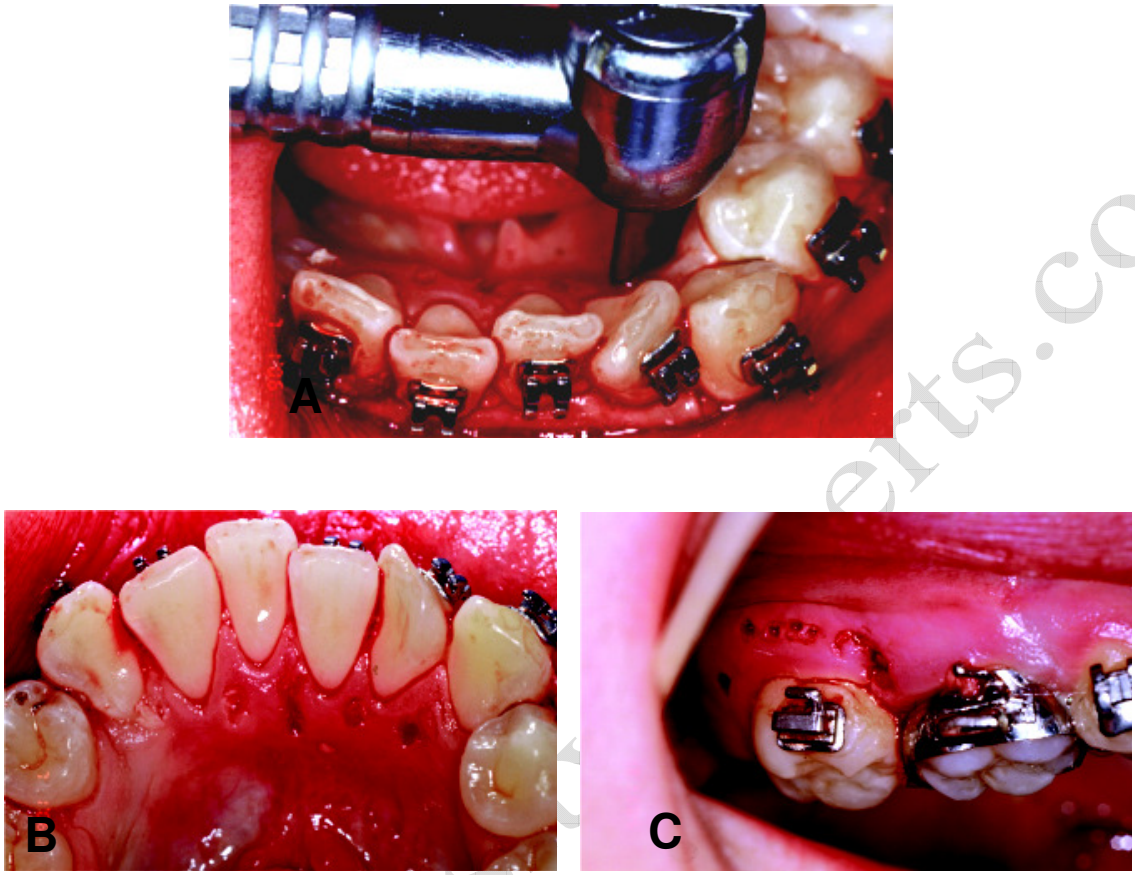


Figure 26 TMP illustrated above can stand as an acronym for "trans mucosal perforation" but to truly understand the dynamics of alveolar tissue engineering one should use it for "trans-mucosal perturbation", *viz.* epigenetic *perturbation* of morphogenetic trajectory to prevent canalization. TMP is an attempt to reinvigorate the tissue healing dynamics, after the Frost-Jee RAP (regional acceleratory phenomenon, or regional osteopenia) has extinguished. The mechanically induced RAP, usually lasting only 6-9 months, can often be prolonged by the addition of viable stem cells. Because orthodontic mechanotherapy may last longer than the RAP, a kind of TMP "booster" is sometimes needed to reassert the induced osteopenic state without resorting to a second surgery. TMP is also an epigenetic perturbation as hMSCs are re-stimulated to continue a novel trajectory to pre-designed alveolus morphology on Waddington's Epigenetic Landscape (see Figure 27). Thereafter, RAP, the production of "daughter cells" (conceivably for 6-9 generations), and stem cell differentiation must be sustained by constantly stressing the alveolus by appliance manipulation.

(A) The technique employs a high speed surgical length #2 round bur with external irrigation. It is driven into the alveolus just past the center of rotation of the lower lateral incisor roots. This is repeated every 1-2 mm circumferentially. (B) The punctate divots in the attached gingiva represent TMP of the lingual cortex of the alveolus to facilitate rapid tipping movement of the incisors. Lower incisor crowding was treated to finish in about a week. (C) TMP also has great utility in accelerating second molar eruption. Sometimes a case is finished only to have a malaligned eruption of second molars delay debonding. Holding all the treated teeth hostage to a recalcitrant second molar is not good practice because it strains patient compliance and increases a time-sensitive bacterial load. So eruption of the second molar should be accelerated easily with TMP.

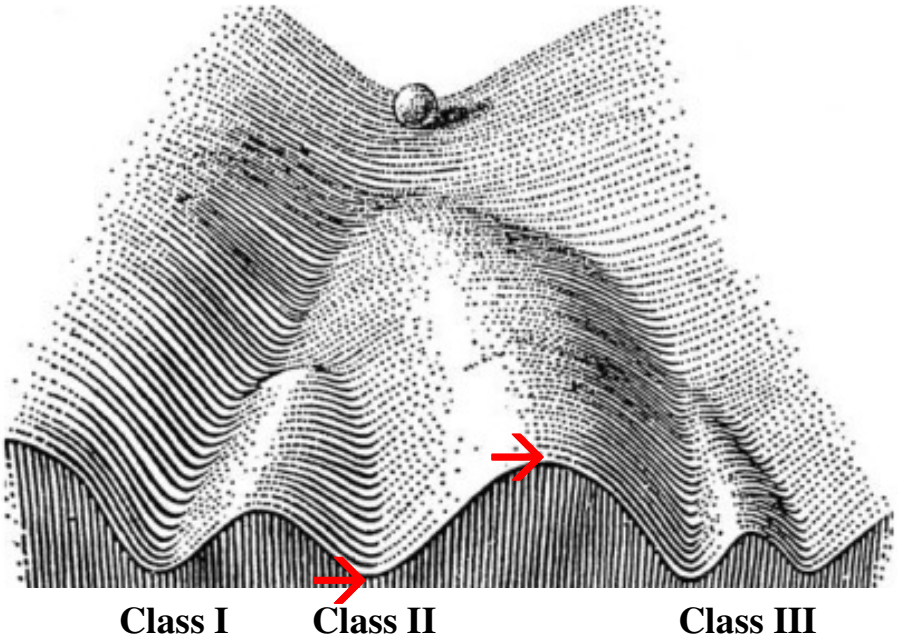
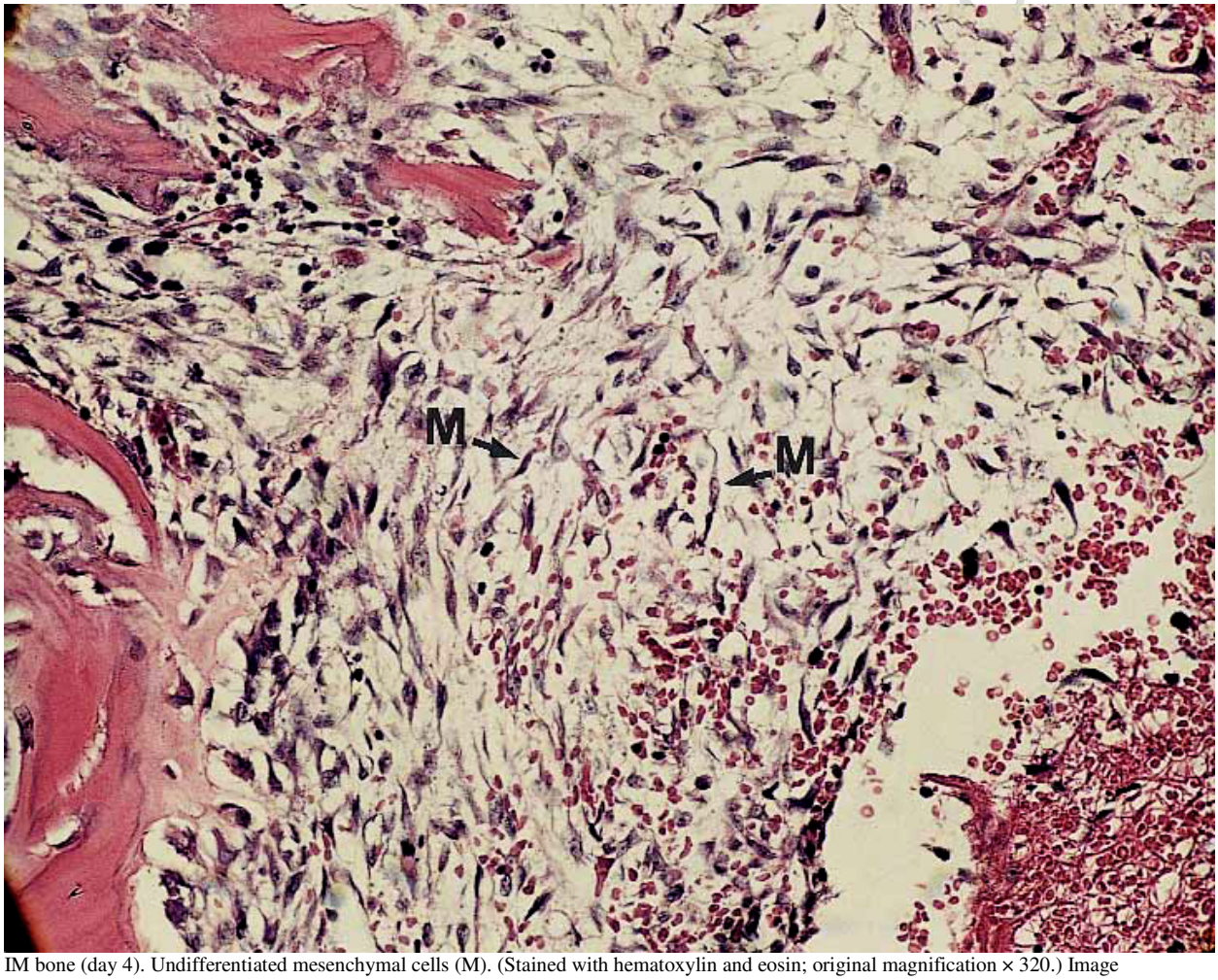


Figure 27 This illustrates the Epigenetic Landscape of Waddington, a visual metaphor and pedagogical tool to explain the interplay of genetic potential, ultimate genetic expression, and environmental "perturbations". The ultimate fate of morphogenesis is generally dismissed as "interplay between nature and nurture" but only the Epigenetic Landscape illustrates exactly how ultimately genetic expression and phenotype are realize. The ball, representing genetic potential, motivated by developmental factors, (in health or disease) moves toward various fates (e.g. Class I, II or III) within stable creases on the landscape. Ridges represent what Waddington called "buffers" to change. The depth of each crease represents a kind of "energy well"(canalization) that affords stability while the height of the ridges represent a kind of "energy of activation" or energy gradient threshold necessary to overcome canalization and achieve new phenotypic fates.

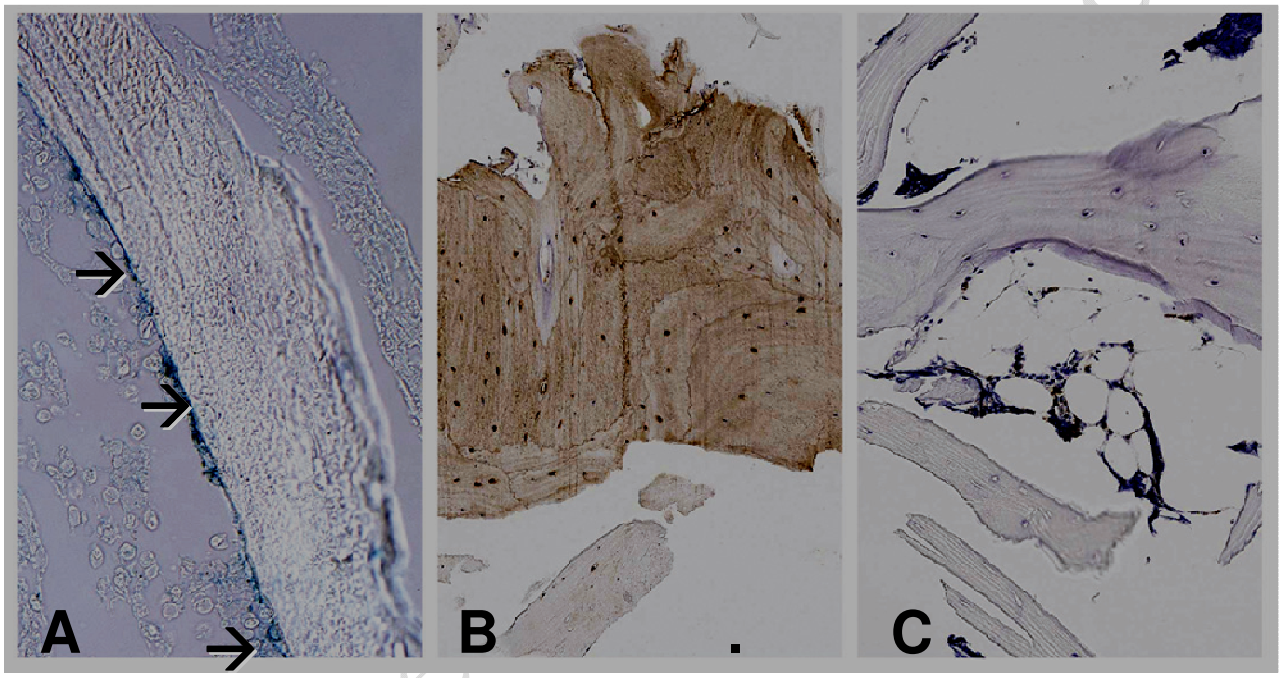
Applying this conceptualization to conventional orthodontics one would note that traditional biomechanics manifestly cannot overcome the energy gradient necessary for treatment stability. Thus, skeletal and dent-alveolar deformities may be described as the end products of simple genetic expression or epigenetic dynamics. Actually they are both as illustrated above. Conventional treatment cannot quite get the "ball over the ridge". Where conventional biomechanics is insufficient, surgical intervention, such as SAD, PAOO and trans-mucosal perforations (TMP or "epigenetic perturbations") can indeed overcome "buffering". This achieves new canalizations necessary for a stable change in phenotype. Clinically, a phenotypic change is expressed as a "stable treatment outcome", e.g. Angle's Class I.

The distance between the red arrows represents the energy of activation that is necessary to overcome "canalization", Waddington's term for the quantum amount of epigenetic perturbation necessary to change from one phenotype to another. The phenotype stability is said to be 'buffered" against change unless canalization can be overcome. Epigenetic influences may be heritable or non-heritable. However, no claim is made herein to Lamarckian concepts.



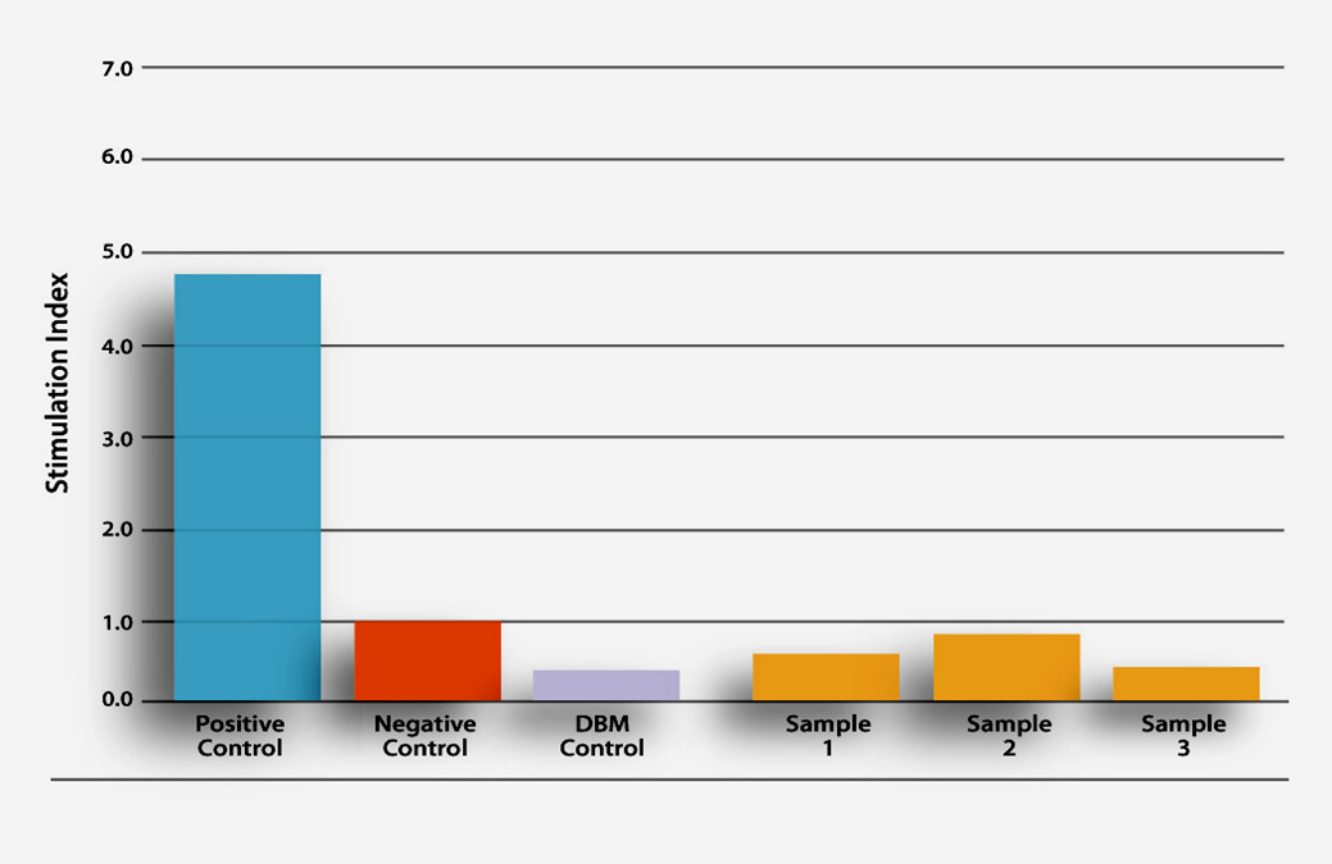
IM bone (day 4). Undifferentiated mesenchymal cells (M). (Stained with hematoxylin and eosin; original magnification × 320.) Image compliments of Dr. A. Bakr Rabie, University of Hong Kong, Republic of China. Used with permission.

Figure 28 Compare the physical appearance of differentiated cells, in Figure 13 with these naturally occurring MSCs (M-arrows) stimulated to form bone in a rabbit animal model.



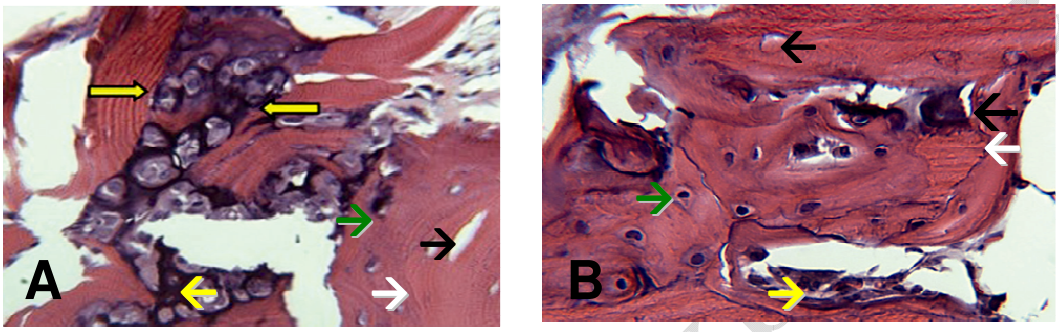
Source: Images compliments of Dr. Ray Linovitz, Orthofix, Inc. San Diego, CA USA Used with permission.

Figure 29 CD stands for "cluster of differentiation" or "cluster of designation". CD is an analytic method used to indentify antigenic markers (determinates). They may be ligands or receptors. Some are not functional in cell signaling but may be related to other cell functions. This image demonstrates two (166 and 45) of 350 CDs are identified in human cells. They are employed here to mark the absence of MSC and HSC. (A) demonstrates CD 166 positive markers for MSC (arrows) Note how the alignment of cells along the viable new bone mimic the spine model samples in Figure 31. (B) Demonstrates osteocalcin stained for osteoprogenitor cells (OPC). Osteocalcin, is a noncollagenous protein which is operative in bone calcification and correleated with bone density. (C) CD 45 marker is negative for hematopoietic cells (HSCs), components of human bone marrow, which are selectively eliminated during allograft processing.



Source: Images compliments of, Orthofix, Inc. San Diego, CA USA Used with permission.

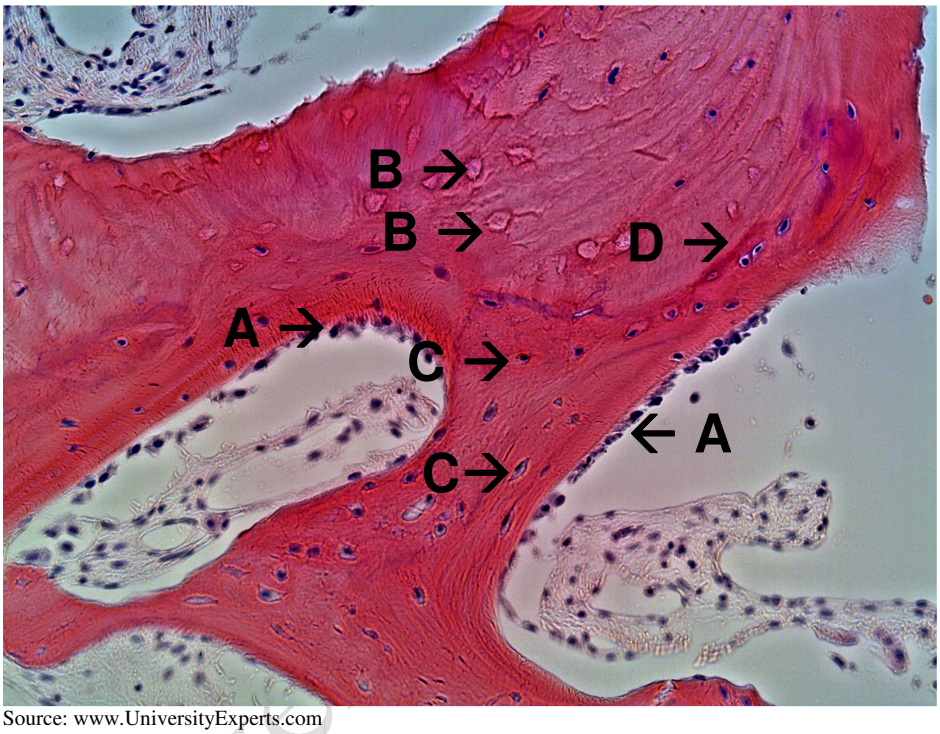
Figure-30 This image demonstrates how commercial sources can ensure that the cells are indeed viable in an allograft matrix.



Source: Nuvasive, Inc. San Diego, CA USA Used with permission.

Source: www.UniversityExperts.com

Figure 31 Note the nearly identical histological architecture of an orthopedic spine fusion model (A and B above) with a specimen taken from a PAOO patient (Patient E.O.) to insure proper bone maturation. Each demonstrates the empty lacunae of DBM (black arrows), reversal lines (white arrows), differentiated osteoblasts, (yellow arrows) and viable bone *de novo* (green arrows). Thus, beyond the merits which stem cells contribute to alveolus phenotype alteration, the alveolus may also serve as a reasonable proxy for long bone and spine surgery analysis.



Source: www.UniversityExperts.com

Figure 32 A high resolution image of the specimen in Figure 31. Note (A) the bone lining cells laying down new bone, (B) empty lacunae of the allograft matrix, (C) viable osteocytes in new bone, (D) reversal lines between layers of new bone and the allograft matrix. This specimen was taken from the labial cortical bone, directly labial to the trajectory of lower incisor tooth movement (0.018 nickel-titanium round wire 2 months after Patient E.O. was treated with PAOO surgery. The evidence above defies the periodontal theory that one "cannot grow bone on a flat surface". Thus, new hypotheses were developed in this paper and the seminal work * to explain what conventional dental theory could not. To our knowledge this is the first publication of successful stem cell-enhanced alveolar orthopedic therapy in the dental literature. This clearly opens interesting vistas for clinical practice since the surgical procedure was executed as an out-patient procedure with only light anxiolytic medication *per os* and local anesthesia (Lidocaine®)

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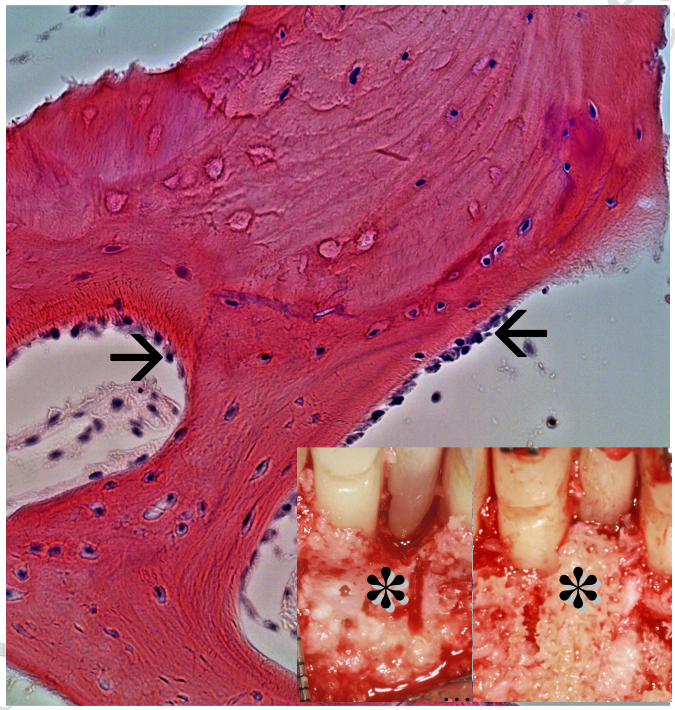
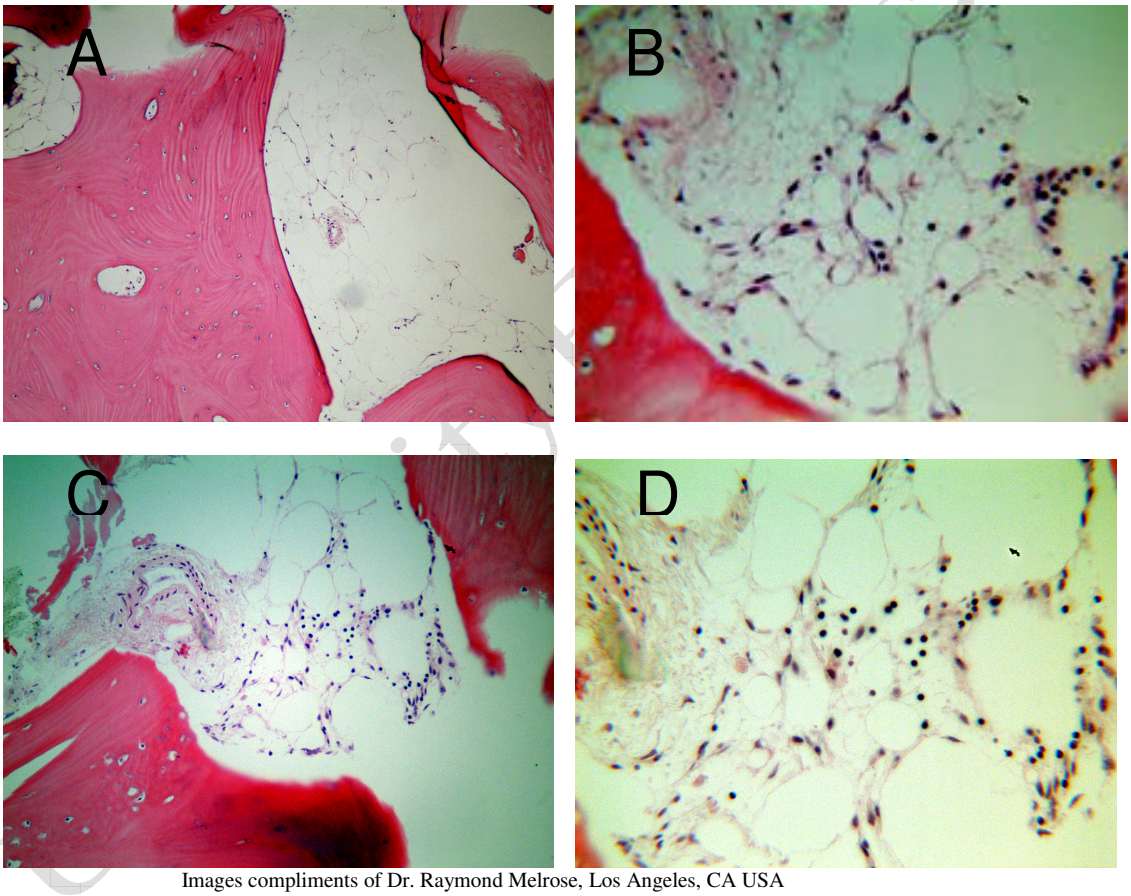
*See: Murphy, N.C., (2006) in vivo Tissue Engineering for Orthodontists: a modest first step. In: *Biological Mechanisms of Tooth Eruption, Resorption and Movement*, Davidovitch Z, Mah J and

Suthanarak S, (Eds.) 385-410.Harvard Society for the Advancement of Orthodontics, Boston, Massachusetts. Available at: www.UniversityExperts.com



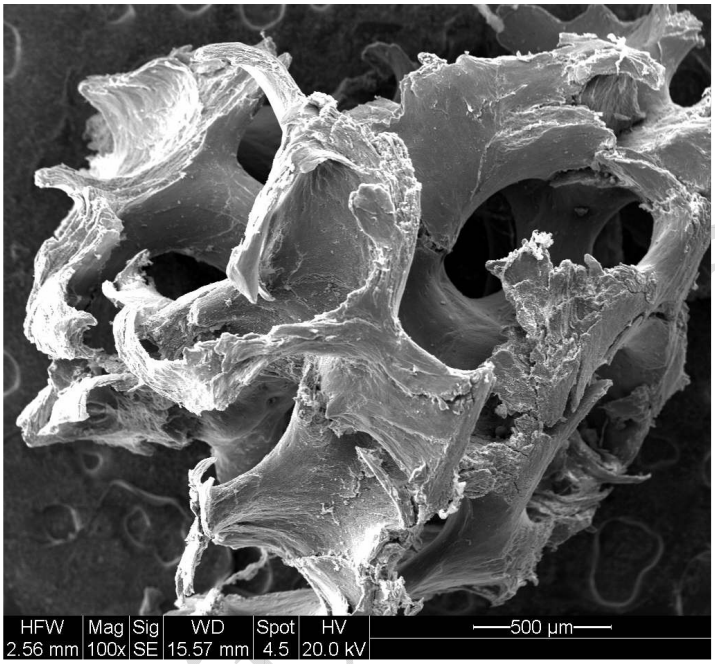
Source: Dr. Neal C. Murphy CWRU, UCLA wwww.UniversityExperts.com

Figure 33 the composite image demonstrates the location from which the tissue sample was taken 2 months after commencement of labial movement of the mandibular incisors. The root of #25 moved labially into the viable cell allograft, reduced clinical attachment loss and presumably induced complete, anatomically normal and functional, periodontal attachment apparatus regeneration ("new attachment"). Immediately after the last suture was tied over the living cell allograft mandibular incisors were moved anteriorly into the graft with the full engagement of a 0.018" nickel-titanium round archwire. The layer of bone forming cells (arrows) should not be conceived as a line, but rather as a kind of "blanket" that covers the entire plane of new bone.



Images compliments of Dr. Raymond Melrose, Los Angeles, CA USA

Figure 34 A standard H& E stained section of the non-implanted (in vitro) viable stem cell allograft taken directly from the cryopreserved container. (A) demonstrates processed bone matrix (empty lacunae) and viable bone (note living osteocytes in lacunae). The spindle shaped cells at the periphery may be undifferentiated stem cells but definitive assessment is not possible in this sample. Note that osteoblasts do not line the edge of the viable bone as they do in Figure 33.



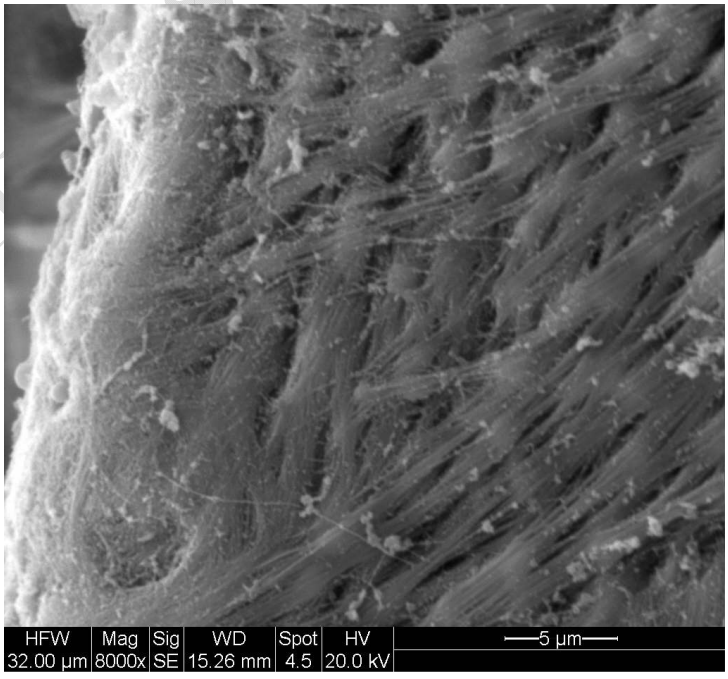
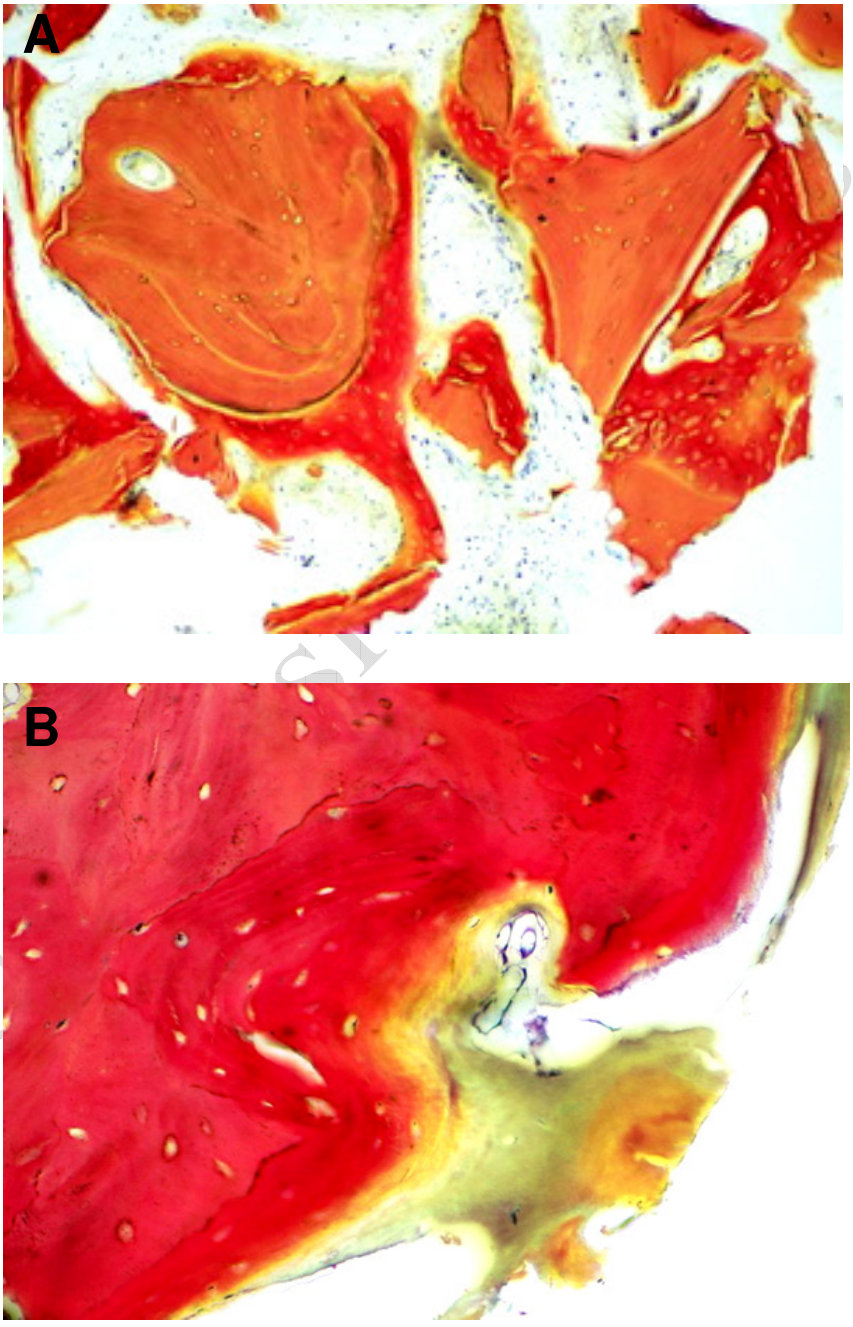


Figure 35 Scanning electron microscopy of the viable cell matrix with stem cells and osteoprogenitor cells. Compare the paucity of cells in the field with the cell replete in vivo sections in Figs 31-34.



Source: Professor Bradley S. McAllister, Assistant Professor, Department of Periodontology, Oregon Health Sciences University and Private Practice, Portland, OR, USA. Used with permission.

Figure 35 Stem Cell Therapy is a *fait accompli* in other areas of clinical dentistry by pioneering clinical researchers in the USA and Canada. Note the consistent and similar histological picture in these histological sections, taken from a human maxillary sinus augmentation sites after 3.5 months, to those in Figures 31-34 taken after 2 months. Representative images are from mineralized core in **(A)** at high (100x) and in **(B)** very high (200x) magnifications. The red-stained tissue is mineralized allograft, with the lighter red being the non-vital bone with no live cells. The newly regenerated bone is darker red, with visible cell nuclei. The green-stained tissue in **(B)** is demineralized allograft, containing neither viable bone, nor cells.

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Notes and Corrections: