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J Dent Res 93(12):1196-1202, 2014

ABSTRACT

Large mandibular defects are difficult to reconstruct with good functional and aesthetic outcomes because of the complex geometry of craniofacial bone. While the current gold standard is free tissue flap transfer, this treatment is limited in fidelity by the shape of the harvested tissue and can result in significant donor site morbidity. To address these problems, *in vivo* bioreactors have been explored as an approach to generate autologous prefabricated tissue flaps. These bioreactors are implanted in an ectopic site in the body, where ossified tissue grows into the bioreactor in predefined geometries and local vessels are recruited to vascularize the developing construct. The prefabricated flap can then be harvested with vessels and transferred to a mandibular defect for optimal reconstruction. The objective of this review article is to introduce the concept of the *in vivo* bioreactor, describe important preclinical models in the field, summarize the human cases that have been reported through this strategy, and offer future directions for this exciting approach.

KEY WORDS: bioengineering, craniomaxillofacial surgery, bone graft(s), bone remodeling/regeneration, clinical studies/trials, tissue engineering.

DOI: 10.1177/0022034514547763

Received June 3, 2014; Last revision July 17, 2014; Accepted July 26, 2014

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In Vivo Bioreactors for Mandibular Reconstruction

INTRODUCTION

While all the components of the human skeleton serve important functional roles, the craniofacial bone plays a particularly essential part in the human psyche. Large mandibular defects result in the loss of functional capacity, such as the ability to masticate, but the accompanying loss of aesthetics can have equally devastating psychosocial complications (Macgregor, 1990; Hickey and Salter, 2006). The current techniques available to surgeons to repair these defects are limited; treatment often requires donor tissue, resulting in additional morbidity, and aesthetic restoration is limited in fidelity by the match of the donor tissue geometry (Table 1). Therefore, new treatment modalities are needed to improve patient outcome. One developing approach is the use of *in vivo* bioreactors to generate autologous bone tissue (prefabricated flaps) to fill mandibular defects. Bioreactors in general are chambers in which biological tissues can be grown. Classically, bioreactors are *ex vivo*, and different conditioning regimes have been explored to optimize tissue growth, as explored in a recent review (Salehi-Nik *et al.*, 2013). However, it is difficult to fully recapitulate a physiological environment under *ex vivo* circumstances. *In vivo* bioreactors are those implanted inside an organism, leveraging the natural regenerative capacity of the body to generate tissue. In this work, we review both preclinical and clinical data available on the *in vivo* bioreactor strategy for mandibular repair (Fig.). This information is synthesized to present goals for the field in further advancing the strategy of *in vivo* bioreactors for the treatment of human patients.

PRINCIPLES OF IN VIVO BIOREACTORS

More than 25 yr after Marshall R. Urist (1965) recognized and isolated what is now known as bone morphogenetic protein, Khouri *et al.* (1991) were the first to implant silicone molds loaded with osteogenic growth factors to generate vascularized bone tissue with specific anatomic geometries ectopically in rats. This landmark study demonstrated the feasibility of harnessing the native regenerative capacity of the body with exogenous signals to generate autologous tissue of prespecified shape. While there have been many attempts to grow large complex tissues *in vitro*, the vascularization and architecture of tissues are very difficult to recapitulate in artificial environments. Therefore, new strategies are being developed to utilize the body itself as a bioreactor to grow new tissue for transplantation to a defect. The *in vivo* bioreactor strategy has been applied for the synthesis of bone to repair musculoskeletal defects. For example, Stevens *et al.* (2005) created an injectable hydrogel capable of functioning as a bioreactor supporting new bone growth when injected underneath the periosteum in a rabbit model. However, geometric precision is less critical in the repair of musculoskeletal defects compared with defects of the

Table 1. Advantages and Disadvantages of Current Repair Strategies

Reconstruction Strategy	Autologous Grafts	Autologous Free Tissue Flaps	Alloplastic Permanent Prostheses	Alloplastic Synthetic Grafts
Advantages	<ul style="list-style-type: none"> • Possess endogenous osteoinductive / osteoconductive factors • Consist of relatively simple procedure 	<ul style="list-style-type: none"> • Established as current gold standard • Result in vascularized tissue 	<ul style="list-style-type: none"> • Result in no donor site morbidity • Have capability of fully recapitulating defect geometry 	<ul style="list-style-type: none"> • Result in no donor site morbidity • Are available in different ceramic compositions
Disadvantages	<ul style="list-style-type: none"> • Result in donor site morbidity • Lack vasculature • Are appropriate only for small defects 	<ul style="list-style-type: none"> • Result in donor site morbidity • Give rise to increased operation room time • Are technically demanding 	<ul style="list-style-type: none"> • Demonstrated to have high risk of infection • Often result in poor soft tissue integration • Carry potential risk of extrusion 	<ul style="list-style-type: none"> • Lack vasculature • Prediction of resorption rates is difficult

facial bone. Therefore, strategies for the generation of craniofacial bone require more control over the shape of generated tissue than what an injectable hydrogel system would allow. While the focus of this review is the use of *in vivo* bioreactors for mandibular tissue, readers are directed to a previous review on the subject of *in vivo* tissue engineering for more musculoskeletal applications (McCullen *et al.*, 2011) and a review with broad craniofacial focus (Torrioni, 2009).

For the purposes of mandibular reconstruction, the *in vivo* bioreactor has been evaluated in many preclinical models and in several patient case reports. Investigators use different combinations of material scaffold, seeded cell populations, and exogenous growth factors to generate autologous prefabricated flaps. There is a large variety of biodegradable scaffold materials, including the broad classes of ceramics and polymers. Ceramics possess strong compressive properties and may be naturally derived (*e.g.*, morcellized autograft) or synthetic (*e.g.*, hydroxyapatite or beta-tricalcium phosphate; Betz, 2002). In addition, ceramics may degrade in the form of free calcium and phosphate ions that may promote future osteogenesis. Nonetheless, some ceramics are limited in degradability. In contrast, polymer-based scaffolds have high tunability of material properties, such as mechanical strength and degradation (Athanasίου *et al.*, 1998). For the purposes of the mandible, however, these scaffolds may be less biomimetic, and many of the common biodegradable polymers degrade into acidic by-products that can inhibit bone formation (Athanasίου *et al.*, 1998). Cells may or may not be seeded onto the scaffold; the most commonly used cell population is bone marrow aspirate from the iliac crest (Orringer *et al.*, 1999; Warnke *et al.*, 2004). Last, exogenous growth factors may

Considerations in Designing In Vivo Mandibular Bioreactor Strategies

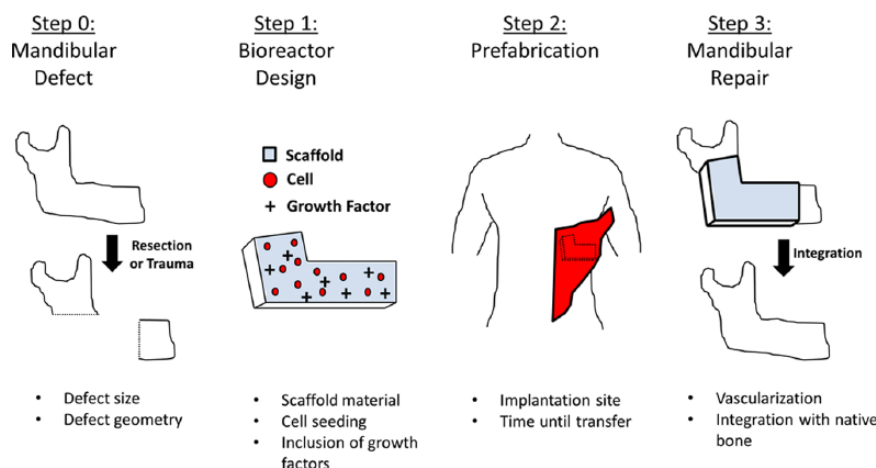


Figure. Schematic of the *in vivo* bioreactor strategy and variables that must be considered at each step. In this example, the latissimus dorsi is used as a prefabrication site for bioreactor implantation.

or may not be added to the bioreactor before implantation. The 2 growth factors that have been most explored are bone morphogenetic protein 2 and 7 (BMP2 and BMP7; Warnke *et al.*, 2004; Heliotis *et al.*, 2006). However, as many patients in need of mandibular reconstruction have a history of craniofacial tumors, there is concern with the use of growth factors in this population; in fact, BMP2 is contraindicated in patients with a history of cancer (Gitelis *et al.*, 2008; Carragee *et al.*, 2011). Ideally, an *in vivo* bioreactor strategy minimizes the use of the following:

- Autologous scaffold, thereby mitigating donor site morbidity
- Seeded cells, as bone marrow aspiration also involves (albeit limited) donor site morbidity and increases approach complexity
- Exogenous growth factors, thereby mitigating the risks accompanying therapy

DESIGN OPTIMIZATION IN PRECLINICAL MODELS

Model Selection

Small Animal Models

For the purposes of this review, small animal models are defined as mice, rats, and rabbits. The use of small animals is attractive, as they are inexpensive to purchase and maintain. The amount of growth factors and scaffold material needed in small animals is also significantly reduced. Not surprising, a number of studies have taken advantage of small animal models. For example, rat mesenchymal stem cells were seeded in a polymer hydrogel and implanted within the dorsum of immunodeficient mice to form mandibular condyle constructs of human proportion. These condyles had both cartilage and bony sections, demonstrating the efficacy of the *in vivo* bioreactor approach in generating complex tissues with multiple components (Alhadlaq and Mao, 2003). An early study in rabbits demonstrated histologically that bone generation in an *in vivo* bioreactor was similar to native bone in architecture and vascularization (Celik *et al.*, 2000). The rat latissimus dorsi has been used in several studies as the site for bioreactor implantation, most often to study the effects of different amounts and types of growth factors (Kusumoto *et al.*, 1998; Roldan *et al.*, 2004; Holt *et al.*, 2005). Ultimately, defects in small animals lack the diffusional challenges presented in defects of clinical relevance. This presents a significant limitation in their use as models; large animals are more appropriate for generating approaches that can be translated for human treatment.

Large Animal Models

Sheep. Regarding large animals, the most prevalent in the field of *in vivo* bioreactors for mandibular reconstruction are sheep, minipigs, and nonhuman primates. One of the earliest of these models was the sheep (Miller *et al.*, 1996). In this model, tissue chambers made of polymethylmethacrylate were filled with scaffold material (typically, morcellized autograft) and placed against the cambium layer of the rib periosteum. Up to 4 of these chambers were implanted per animal on alternating ribs. Vascularized bone tissue grew into the chamber from the periosteum and ultimately took the shape of the chamber. In this manner, tissues of complex geometry and clinically relevant size were generated (Miller *et al.*, 1996; Cheng *et al.*, 2009). Using this model, investigators determined optimal prefabrication time (Cheng *et al.*, 2005) and optimal implantation site (Brey *et al.*, 2007) and investigated the use of biodegradable polymers as scaffold to mitigate the need for autologous donor tissue (Thomson *et al.*, 1999). This strategy was translated into the clinic in a human patient (Cheng *et al.*, 2006). In the future, it is envisioned that this periosteal *in vivo* bioreactor will be translated in combination with the 2-stage mandibular reconstruction approach (Atala *et al.*, 2012). In this strategy, a space maintainer is inserted at the time of mandibular resection. The space maintainer acts as a template for soft tissue regrowth, maintains facial contours, and prevents scarring within the defect space (Henslee *et al.*, 2014). It is worth noting that others have also used the sheep for prefabricated mandibular flap generation, with implantation in the latissimus dorsi (Kokemueller *et al.*, 2010; Kokemueller *et al.*, 2013).

Minipig. Another well-established *in vivo* bioreactor large animal model is the minipig. In this model, titanium cages were loaded with bovine hydroxyapatite (Bio-Oss) as a scaffold with the addition of BMP7. These constructs were implanted in the latissimus dorsi of minipigs for the generation of ossified tissue (Terheyden *et al.*, 1999). This prefabricated flap was transferred into a minipig mandibular defect with great success. In fact, when the contralateral mandible was treated with primary reconstruction *via* the same ceramic scaffold and growth factor regimen, the prefabricated flap resulted in bone of better quantity and quality (Terheyden *et al.*, 1999; Terheyden *et al.*, 2001). As evaluated by mechanical testing, these autologously generated tissues had similar compressive properties to native mandibular bone (Warnke *et al.*, 2006a). The strategy applied in minipigs was also clinically translated in a human patient (Warnke *et al.*, 2004; Warnke *et al.*, 2006a).

Nonhuman primates. Nonhuman primates have also been used as a model for the development of *in vivo* bioreactors—specifically, to compare (1) reconstruction of a mandibular defect with tissue from a bioreactor implanted in the latissimus dorsi treated with BMP2 with (2) primary defect repair with BMP2. In this model, the *in vivo* bioreactor approach demonstrated better bone formation in both quantity and quality (Zhou *et al.*, 2010). While primates most closely approximate the human condition, this research is very costly and raises complex ethical questions.

Prefabrication Site

One important consideration for the *in vivo* bioreactor strategy is the site of bioreactor implantation. Implantation in different environments may result in the recruitment of different types of factors, such as blood vessels, nerves, progenitor cells, and signaling proteins. An ideal ectopic site also minimizes inflammation, pain on implantation, and complications upon harvest (McCullen *et al.*, 2011; Miller *et al.*, 1996). For the purposes of bone tissue engineering, bioreactor implantation against the periosteum has been demonstrated as a successful environment for ossified tissue generation (Miller *et al.*, 1996; Thomson *et al.*, 1999; Cheng *et al.*, 2005; Stevens *et al.*, 2005; Brey *et al.*, 2007; Cheng *et al.*, 2009). In a study in which identical tissue chambers (filled with morcellized bone graft) were implanted against either the rib periosteum or the fascia of the latissimus dorsi muscle in sheep, both sites were able to generate vascularized tissue. However, the intramuscular implants had significant graft resorption and resulted in primarily fibrovascular tissue. Chambers implanted against the periosteum had active bone formation with increased calcified tissue area (Brey *et al.*, 2007). Nevertheless, with the addition of exogenous growth factors, intramuscular implantation can result in ectopic bone formation within a bioreactor (Kusumoto *et al.*, 1998; Roldan *et al.*, 2004; Geuze *et al.*, 2012).

Prefabrication Time and Geometry

For the formation of ossified tissue in particular, the time of implantation has a significant impact on the tissue quality. The

sheep rib periosteum model has demonstrated that there is a specific window at which ossified tissue is at peak quantity and quality (6-9 wk), after which resorption occurs within the bioreactor (Cheng *et al.*, 2005; Cheng *et al.*, 2009). The shape of the bioreactor chamber, so long as it can fit within its prefabrication site, appears to be flexible and can be designed to conform to the geometry of the defect (Miller *et al.*, 1996; Cheng *et al.*, 2009). However, the composition of the bioreactor chamber itself may play a significant role in tissue development. In the minipig model, chambers constructed from biodegradable polymers were not able to maintain their predefined shape over the implantation period (Warnke *et al.*, 2006a). As control over geometry is one of the key advantages to the *in vivo* bioreactor strategy, this suggests that chambers should be composed of nonbiodegradable materials, such as titanium or polymethylmethacrylate.

Scaffold Material, Growth Factors, and Seeded Cells

In addition to prefabrication site and implantation time, the 3 aspects of the classic tissue engineering paradigm (cells, chemical cues, and scaffolds) are also critical in the design of the *in vivo* bioreactor strategy. In preclinical models, a variety of combinations have been demonstrated to promote the growth of a prefabricated tissue flap. For scaffold material, investigators have experimented with morcellized autograft, decellularized bone graft, collagen, biodegradable polymer, hydroxyapatite, beta-tricalcium phosphate, and various biphasic ceramics (Kusumoto *et al.*, 1998; Thomson *et al.*, 1999; Holt *et al.*, 2005; Eweida *et al.*, 2011). In one study, *in vivo* bioreactors filled with different scaffold materials (morcellized autograft, decellularized autograft, and empty chambers) were implanted against sheep rib periosteum for comparison (Cheng *et al.*, 2005). Morcellized autograft generated significantly more bone tissue than any of the other groups. Otherwise, as there have not been additional studies in which different scaffold materials have been compared within the same animal model, there is little evidence regarding which scaffold material is optimal for the generation of ossified tissue. While it has been suggested that osteogenic growth factors are required for bone tissue formation in intramuscular implants, the addition of exogenous growth factors in bioreactors implanted against the periosteum is not required for bone growth (Miller *et al.*, 1996; Thomson *et al.*, 1999; Cheng *et al.*, 2005; Brey *et al.*, 2007; Cheng *et al.*, 2009). Last, while cell seeding is an absolute necessity for *in vitro* approaches, cells can be locally recruited with *in vivo* bioreactors (although bone marrow aspirates can also be used to seed the bioreactors upon implantation; Cheng *et al.*, 2005; McCullen *et al.*, 2011). The lessons learned from preclinical models have resulted in the translation of this technology to human patients.

HUMAN CASE REPORTS

As an exciting development in the field, 5 different *in vivo* bioreactor approaches in human patients for mandibular reconstruction have been reported in the literature. The first of these cases occurred in 1990 (although it was not reported until 1999).

In this landmark case, a patient who had lost the mandible due to recurrent ameloblastoma was treated with a mandibular-shaped Dacron-polyurethane tray packed with autologous bone graft and exogenous growth factor (Orringer *et al.*, 1999). This tray was implanted in the fascia above the scapula and retrieved after 4 mo (skin grafting over the retrieval site was required). The patient's lower lip was also reconstructed with strips from the harvested tensor fasciae latae. While the patient tolerated the procedure well, the reconstruction did not permit oral feeding and was not sufficient to support dental implants. Eventually, a revision was performed to improve function as well as augment the bone with grafts for the insertion of dental implants. The patient never regained the ability to swallow solids and unfortunately passed away due to disseminated disease.

The next reported case was presented by Warnke *et al.*, the same group that developed the minipig model (Terheyden *et al.*, 1999; Terheyden *et al.*, 2001; Warnke *et al.*, 2006a). In this case, a patient with a history of oral tumor was treated with a titanium cage filled with Bio-Oss and BMP7 (Warnke *et al.*, 2004). This bioreactor was implanted intramuscularly within the latissimus dorsi. Bone growth and remodeling within the bioreactor were confirmed before harvest by use of skeletal scintigraphy. After 7 wk, the prefabricated flap was harvested and transferred along with the accompanying titanium mesh. Scintigraphy was repeated after transfer and revealed further bone remodeling, indicating integration with native bone. The patient had restored aesthetics and function and regained the capacity to enjoy solid foods (Warnke *et al.*, 2006b). The authors of the study particularly comment on the sociopsychological effects of this treatment on the patient, noting that the patient's "mood turned from one previously of depression and suicidality to one of excitement and optimism." Ultimately, after 13 mo, the titanium mesh fractured, and the soft tissue overlying the construct failed. This resulted in infection and necrosis of the transferred bone. Two subsequent revisions were performed, and eventually the patient died as a result of cardiac arrest.

Using the same strategies as developed in the sheep periosteal model, Cheng *et al.* (2006) augmented a mandibular reconstruction with an *in vivo* bioreactor approach. In brief, a patient presented with numerous reconstructions of both hard and soft tissue to restore function after removal of an oral squamous cell carcinoma. However, these reconstructions did not generate mandibular bone of sufficient height to accommodate dental implants. Thus, a polymethylmethacrylate chamber was filled with harvested autograft and implanted against the periosteum of the iliac crest. After 8 wk, the tissue was harvested, and donor periosteum was sutured to mandibular periosteum to reestablish blood supply. The patient eventually died of hepatocellular carcinoma, but the transferred tissue was functional and retained dental implants at 16 mo.

In an effort to reduce donor site morbidity, Heliotis *et al.* (2006) designed an *in vivo* bioreactor approach without the use of harvested autologous bone or bone marrow cells. BMP7 was added to hydroxyapatite blocks and implanted in the pectoralis major muscle of a patient who had suffered from oral squamous cell carcinoma. After 3.5 mo, bone scintigraphy revealed bone formation in the construct. After 6.5 mo of implantation time,

Table 2. Case Report Strategies and Outcomes

Prefabrication Site	Scaffold Material	Growth Factors	Seeded Cells	Outcome	Author
Scapular fascia, 4 mo	Dacron-polyurethane cage + autograft	BMP (undefined)	Bone marrow aspirate	N/A; died of recurrence after ~2 yr	Orringer <i>et al.</i> , 1999
Latissimus dorsi, 7 wk	Titanium cage + decellularized xenograft	BMP7	Bone marrow aspirate	Infection and revision, 13 mo	Warnke <i>et al.</i> , 2004; Warnke <i>et al.</i> , 2006b
Iliac crest periosteum, 8 wk	Autograft	None	None	N/A; died of unrelated cancer after ~ 16 mo	Cheng <i>et al.</i> , 2006
Pectoralis major, 6.5 mo	Hydroxyapatite	BMP7	None	Infection, 5 mo	Heliotis <i>et al.</i> , 2006
Latissimus dorsi, 6 mo	Beta-tricalcium phosphate + autograft	None	None	N/A, 13 mo	Kokemueller <i>et al.</i> , 2010

the construct was harvested, along with muscle tissue, for transfer into the mandibular defect. The tissue was covered with a split skin graft. Unlike previous studies, a biopsy was taken from the tissue at the time of transfer. The transferred construct was 17% bone, 37% hydroxyapatite, and 46% fibrovascular tissue. After 5 wk, the transferred tissue became infected, and the flap was removed. This case is notable for its histologic analysis of prefabricated clinical tissue and lack of need for donor tissue in flap generation.

Finally, a recent case was reported in which cylinders of beta-tricalcium phosphate were loaded with cells and morcellized autologous bone graft from the iliac crest (Kokemueller *et al.*, 2010). Four of these cylinders were implanted in the latissimus dorsi of a patient who had been suffering from chronic mandibular osteomyelitis requiring resection. After 6 mo, these cylinders were harvested, shaped by piezoelectric surgery, and transferred to the defect. Before transfer, vascularization of these cylinders was confirmed by angio-computed tomography. Gaps between cylinders were filled with additional iliac crest graft. At 12 mo of follow-up, the reconstructed mandible was still viable.

Table 2 summarizes the use of autologously harvested scaffold, bone marrow-derived seeded cells, and exogenous growth factors in each case. These *in vivo* bioreactors were implanted for a minimum of 7 wk and maximum of 6.5 mo before transfer. All approaches resulted in at least temporarily successful reconstruction (although it is possible that additional failed approaches have gone unreported). In 2 out of 5 of these cases, the reconstructed mandible failed or otherwise required significant revision. It is worth noting that in both cases, infection was involved with failure.

CLOSING REMARKS AND FUTURE DIRECTIONS

These initial results demonstrate early promise in the use of an *in vivo* bioreactor strategy for the reconstruction of large mandibular defects, although significant research is still required. While mandibular reconstruction is difficult due to complex geometry, *in vivo* bioreactors can produce bone tissue with dimensions of high fidelity and mitigate donor site morbidity.

Preclinical models, including nonhuman primates, have demonstrated that reconstruction performed with prefabricated tissue compared with primary definitive repair resulted in higher quantity and quality. Case reports in the literature have demonstrated some short-term efficacy of these therapies in human patients. However, as 2 of the 5 reported cases resulted in tissue failure, it is clear that more study needs to be done for this approach to be performed in the clinic on a regular basis.

In regard to preclinical data, small animals have limited utility in investigating the generation of tissue for reconstruction of large mandibular defects due to the lack of diffusional challenge to tissue growth. Out of the large animal models presented, the 2 most established are the minipig latissimus dorsi and sheep periosteal implant models. These 2 models represent both the intramuscular and periosteal implantation strategies that have been used in the clinic to generate tissue for mandibular repair. As these models have been used to compare factors such as implantation time, scaffold material, chamber material, chamber dimensions, and prefabrication *versus* primary reconstruction, it would be beneficial for future work to continue to make use of these well-established models.

For future preclinical work, it would advance the field to explore the effects and necessity of cell seeding on tissue growth. In addition, synthetic ceramic particles should be compared with morcellized autologous graft to potentially reduce the need for any harvested donor tissue. As some biphasic ceramics have been described as osteoconductive and osteoinductive, it may be possible that the use of these scaffolds may circumvent the need to use autologous morcellized bone. In addition, more preclinical studies need to demonstrate tissue transfer into mandibular defects. As the average time to failure in the human reported cases was approximately 9 mo, the long-term efficacy of prefabricated tissue needs to be examined in preclinical models as well.

Despite the available preclinical studies, the ideal implantation site for tissue generation remains unclear. Intramuscular sites facilitate relatively easy transplantation and allow for constructs of large volume to be implanted. However, intramuscular implantation commonly requires the addition of exogenous osteogenic growth factors to produce ossified tissue; this may be

concerning for use in patients with a history of oral cancer and thus introduces additional regulatory hurdles in clinical translation. While the periosteum is a deeper implantation site with less available volume, ossified tissue can easily be generated without any additional growth factors. In addition, infection played a role in both reported cases of failure in the *in vivo* bioreactor approach. The role of antibiotic delivery, especially with new developments in local release, is an important consideration and should be emphasized while moving ahead.

Ultimately, one of the largest barriers to universal translation of the *in vivo* bioreactor strategy for mandibular repair is the lack of a unified approach, as illustrated by the variety of factors in Table 2. Choice of scaffold, inclusion of growth factors and seeded cells, prefabrication site, and prefabrication time are all variables in both preclinical and clinical cases. Based on these case reports, a single approach may be developed and applied in a clinical study. After this point, individual variables could be examined and tuned to further optimize outcomes. Until a single approach is rigorously applied in a clinical study, the strategy lacks the evidence needed to impact the field.

In conclusion, the *in vivo* bioreactor has seen limited success in a small number of case reports. In these select patients, at least temporarily, there was restoration of aesthetics and function. With the developments in areas such as growth factor delivery and synthetic scaffolds, new technologies may enable greater success in the *in vivo* bioreactor strategy. The face plays an essential role in the sense of self; we look forward to the advancement of therapies to restore the mandible even in the most difficult of cases.

ACKNOWLEDGMENTS

This work was supported by the U.S. Army, U.S. Navy, National Institutes of Health, U.S. Air Force, VA, and Health Affairs to support the AFIRM II effort, under award W81XWH-14-2-0004. The U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014, is the awarding and administering acquisition office. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the Department of Defense. In addition, A.M.T. would like to thank the Baylor College of Medicine Medical Scientist Training Program (National Institutes of Health T32 GM007330) and the Barrow Scholars Program. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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