Standard Review Article

OPEN

Basic science review of transcutaneous osseointegration: current status, research gaps and needs, and defining future directions

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Abstract Basic science research is vital for advancing the emerging field of bone-anchored limb replacement (BALR), or osseointegration (OI). This article discusses clinical challenges for BALR/OI, summarizes current basic science research regarding those challenges, identifies research gaps, and proposes future directions. OI research draws from related fields such as orthopaedic implants and dentistry. There is a need for small animal models to study critical questions related to osseointegration, including OI implant-associated infections. Small animal models are also critical to ensuring safety and efficacy of novel treatments in this vulnerable population. Key issues include infection prevention through implant surface modifications, biofilm-targeting technologies, and antimicrobial advancements. The skin–implant portal, unique to BALR, also poses significant challenges. Research on skin attachment and inflammatory processes is crucial. Noninfectious inflammatory loosening of implants, though infrequent, needs further investigation. This review emphasizes the need for collaborative efforts to develop effective preclinical models and innovative infection prevention strategies. Addressing these challenges is essential for optimizing patient outcomes and advancing this emerging field.

Keywords: bone-anchored limb replacement, osseointegration, basic science, skin–implant, infection

1. Introduction

Bone-anchored limb replacement (BALR), also known as osseointegration (OI) for amputation reconstruction, is a field that has had clinical viability over 30 years, although the number of patients treated during that period was relatively low, with an estimated 4000–5000 patients. As an emerging field with limited numbers of patients, basic science, particularly animal models, offers a valuable alternative to clinical research, which often requires large numbers of patients. Animal models are particularly important in testing emerging therapies that have potential risk to already vulnerable patients. Advancing basic science research will be critical to optimize outcomes for an increasingly diverse population of patients.

This article highlights several of the most immediately relevant clinical challenges for BALR/OI, summarize the current state of basic science research, identify key research gaps and needs, and propose future research directions to help inspire and prioritize basic science efforts in the field. Much of BALR's basic science foundation is drawn from closely related fields of orthopaedic implants and implants used in plastic surgery, otolaryngology, and dentistry. The shared reliance on successful integration of various tissues (bone, skin, muscle) and the need to prevent

Leah Gitajn received consulting fees from Stryker and Paragon28. She also has a leadership or fiduciary role in the OTA program committee and AO research committee. Dr. Hsu reports consultancy for Globus Medical and personal fees from Smith & Nephew speakers' bureau. Kyle Potter has a CDMRP PRORP grant/contract with DoD-USUHS Restoral. He also has consulting fees with Integrum and Signature. Danielle Melton has DoD contract OP220013 and CDMRP Grant OR210169. She also has consulting fees for Paradigm Medical Director and has received payment for lectures at the State of the Science Conference on Osseointegration. Danielle Melton has received payment for expert testimony while acting as a consultant and expert witness in multiple cases. She has received support from Amputee Coalition BOD to travel and attend meetings. She has participated in the Data Safety Monitoring Advisory Board for External Advisory Panel for Limb Loss Prevention Registry. Danielle Melton has a leadership or fiduciary role in METRC Executive Council, Amputee Coalition Board of Directors, and in Catapult Board of Directors. Jason Stoneback reports royalties from AQ Solutions as well as consulting fees from AQ Solutions and Smith and Nephew. He reports payment for lectures from Smith and Nephew and AQ Solutions. Jason Stoneback states he has received payment for expert testimony in multiple cases. He notes he has received support to travel and attend meetings from Smith and Nephew and AQ Solutions. He reports planning a patent for a Rotational Intramedullary Nail. Jason Stoneback states he is the secretary for ISPO Special Interest Group for Bone-Anchored Limbs and is a board member for Justin Sports Medicine Team Annual Conference. He also reports stock with Validus Cellular Therapeutics. Robert Rozbruch reports consulting fees from Nuvasive and J&J. He also reports having stock with Osteosys. Jason Souza is a paid consultant for Balmoral Medical, LLC, Checkpoint, Inc, and Integrum, Inc. The remaining authors declare they do not have any conflicts of interest. The remaining authors declare they do not have any conflicts of interest.

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This manuscript did not receive any funding.

Members of the Global Collaborative Congress on Osseointegration (GCCO) are included in Appendix 1 at the end of the article.

OTAI (2025) e367

Received: 21 October 2024 / Received in final form: 11 November 2024 / Accepted: 3 December 2024
Published online 7 March 2025

http://dx.doi.org/10.1097/OI9.0000000000000367

infection allow established findings in related fields to inform osseointegration-specific research questions and models. This article addresses key topics including infection prevention (implant surface modification, biofilm-targeting, and emerging antimicrobial technologies), drawing on relevant studies from related fields. In addition, we will explore BALR-specific challenges, such as the skin–implant portal/interface (or skin penetration aperture), small animal models of BALR/OI, and the infrequent complications of noninfectious inflammatory (or aseptic) loosening.

2. Preclinical Animal Models for OI

2.1. Current Status

Many years of pioneering and innovative OI research have been published by Brånemark et al. Most of these studies have been performed in rabbits toward the goal of characterizing and optimizing implant surface modifications and biomechanical properties to enhance overall OI and prosthetic function.[1–4] These models use an "internal" amputation approach with multiple holes being drilled into long bone metaphyses, thus ultimately lacking the characteristic bone–skin–implant interface of bone-anchored transdermal prostheses, a defining and critical characteristic of BALR/OI. As an orthopaedic and implant infection model, rabbits have emerged as a promising species[5–7] because experimental infectious processes are reliable and reproducible and the rabbit immune system is sophisticated and relevant to human physiology.[8] Rabbits have been used considerably in osteomyelitis research and are a well-established model, and thus, methods for creating repeatable localized infection have been validated.[9–11] They have also been critical in experimental research of basic implant infection susceptibility.[12,13] While the groundwork and need are clear, there remains no small animal model to investigate OI implant-associated infection. Without a well-characterized model, preclinical studies to address infection lack safety and efficacy data, as well as the mechanistic data required by the FDA during certain clinical trial phases. OI human trials are often limited by small sample size, further demonstrating the need for robust animal models. Current work must continue in collaboration between research, veterinary, and surgeon scientists to develop and establish repeatable animal model methods to undertake this infection challenge.

2.2. Gaps, Needs, and Next Steps

Large animal models have been explored[14,15] but lack the bone–implant interface because replicating the amputation and the prosthetic needs of larger animals remains challenging from an animal welfare and budgeting perspective. Limited large animal studies with a true amputation and transcutaneous device have been performed,[16,17] but outcomes were not focused on infection or the skin penetration aperture. An established, repeatable animal model that reproduces all characteristics of BALR must exist to advance much of the basic science research outlined further. Small animal models represent a top priority, as costs and robust sample sizes may be more manageable. Large animal models can serve a specific purpose once a technology or method proves effective in small animal models and can provide immediate preclinical data before human subjects testing but will remain limited by costs, sample size, and ability to produce mechanistic data.

3. Infection Prevention and Treatment

Infection remains a substantial challenge for most orthopaedic implants. Implants can act as conduits for microbes and pathogens, increasing the risk of infection by providing a surface for bacterial adhesion and biofilm formation. This phenomenon has been recognized for over 60 years, with studies demonstrating that the presence of a foreign body significantly reduces the bacterial load required to establish an infection. An early study reported that the presence of an implant increased bacterial virulence 10,000-fold in healthy participants.[18] Subsequent research has confirmed this finding, establishing the ID_{50} in multiple pathogens in response to various types of materials in clinical use.[19] This explains the high prevalence of implant-associated infections and the need for aggressive interventions, such as systemic antibiotics, revision surgery, implant removal, and staged procedures. As infection is one of the primary challenges faced by OI amputees, much basic science research has been directed toward this issue. Infection remains the most commonly reported adverse event in osseointegration literature. We will outline several approaches to prevent and combat implant-associated infections currently being investigated in basic science research.

3.1. Current Status

3.1.1. Implant Surface Modifications. Modifications to the implant surface can promote good primary osseointegration while also repelling bacteria, thus offering prevention against infections. Although the surface roughness itself can have microscale or nanoscale characteristics, including the innate geometry, additional modifications can improve biocompatibility properties toward the goal of preventing infection. Some surface modifications involve control of nanotopography and microtopography using surface complexity created by the addition of nanoscale or microscale molecules to resist bacterial colonization and promote timely tissue integration. Other modifications include surface coatings such as biomaterials and biomimetics that offer anti-infective strategies, osteoinductive/osteoconductive materials, and direct functionalization of antibiotics to implant surfaces. Some biomaterials are close to clinical use, such as those containing antimicrobial and antibiotic substance, bactericidal, and adhesion-resistant coatings. Others are in preclinical testing, such as nanomaterials and biomaterials targeting biofilms and immune-modulatory systems. A thorough review of anti-infective biomaterials has been published, and the authors refer to Campoccia et al[20] for a more comprehensive discussion.

In the clinical setting, implant surface morphology modifications are showing promise in reducing the risk of infection[21–24] by preventing bacterial adhesion through wettability, antifouling, and surface roughness properties. One such method of limiting bacterial interactions is by altering the hydrophilicity and hydrophobicity of an implant surface. In a study comparing titania nanotube arrays with differing superhydrophobic and superhydrophilic properties, fewer bacteria were found to adhere to the superhydrophobic surface compared with the unmodified titanium surface.[25] Although unable to completely repel bacteria, findings indicated that superhydrophobic surfaces greatly reduced the risk of infection by preventing biofilm formation of either Gram-positive or Gram-negative bacteria.[26] The effects of this on bone integration of the implant is not known either as well.

BALR implants are primarily composed of titanium or titanium alloys because of robust biomechanical factors and high

resistance to corrosion. As titanium is generally recognized as an inert material, some methods aim to enhance host cell attachment and/or directly inhibit bacterial attachment. Titanium nanotube arrays (TiO_2) are a surface topography modification that can be achieved by anodization. When TiO_2 nanopillars are designed with mixed height and density, bacterial membranes are stretched across nanopillars and ultimately rupture. In addition, studies have shown that TiO_2 nanotubes can promote adhesion, proliferation, and differentiation of osteogenic progenitors and deposition of osteoblast plaque proteins. A separate study validated these findings by demonstrating increased expression of vinculin, a focal adhesion protein, by mouse osteoprogenitor cells on an anodized TiO_2 surface compared with a nontreated surface.[27] This study also indicated that the spreading and dispersal of both osteoblasts and bacterial cells on the TiO_2 surface occur over a larger area compared with the control surface, which in turn facilitates the proliferation and differentiation of osteoblast cells while preventing bacterial colonization.[27–29]

3.1.2. Biofilm-Targeting Technologies. Biofilm provides protection for the bacteria or microorganisms living within its robust protective matrix architecture, and biofilm disruption strategies allow access of antimicrobial agents to directly kill these individual microbes.[30] Preventing and disrupting biofilms and their matrices is an attractive infection prevention and treatment approach, yet translation has been limited. One disruption method uses enzymes that degrade specific constituents of the electropolymeric substances (EPSs) of biofilms. This allows targeting of 2 key biofilm protective components responsible for providing structure, nutrient supply, and facilitating gene transfer between bacterial cells: environmental DNA (eDNA) and polysaccharide components (eg, polysaccharide intercellular adhesin [PIA]).[31–33] Dispersin B and DNase I are 2 enzymes that have been shown to perturb the EPS by disrupting polysaccharides and eDNA, respectively.[34,35] However, biofilm disruption leaves microbes in a planktonic state that can allow bacteria to disperse, spreading the infection if the microbes are not killed immediately. Thus, biofilm-targeting technologies must be used in conjunction with antimicrobial agents and strategically prime established biofilms for bactericidal activity rather than directly killing cells. Some basic science studies have elucidated methods to couple enzyme activity with antibacterial agents into so-called nanozymes, such as complexing DNase I with peroxidase-like activity.[36] A biofilm-disrupting antibody has demonstrated effectiveness in preclinical biofilm-mediated implant infection models[37] and safety in a Phase 1 study in prosthetic joint infections.[38]

3.1.3. Alternatives to Antibiotics and Traditional Antimicrobials. Traditional antibiotic and antimicrobial approaches are susceptible to drug resistance, potential toxicity, and a narrow spectrum of activity. Alternatives to address these drawbacks have begun to emerge and rely on technologies that are nonleaching, noneluting, and durable. One study coupled a highly nonfouling betaine modification on a titanium implant surface and demonstrated more than 8 weeks of controlled release of chlorhexidine and a greater than 99.9% reduction in bacterial adherence even after serum exposure.[39] Similar findings were described when a greater than 98% reduction of proteins, microbes, and mammalian cells (which deposit their own bacteria-attracting proteins) was observed on the surface of a nonleaching polymeric sulfobetaine (polySB)–-modified implant.[40] This polySB technique reduced accumulation of thrombotic material (>99%) for up to 60 days after serum exposure by coordinating free and bound water molecules to create a hydrophilic surface. Other studies have used a layer-by-layer (LbL) method to assemble multilayer films incorporated with antimicrobial peptides (AMPs). AMPs are broad-spectrum peptides and a component of the eukaryotic innate immune system used in first-line defenses against invading pathogens. AMPs are broad spectrum and active against drug-resistant bacteria, while avoiding contributing to further resistance.[41–43] An antifungal cationic AMP incorporated into an LbL film demonstrated strong action against various Gram-positive and Gram-negative bacteria and fungi, while importantly no drug was found to elute from the films.[44,45] Another AMP known to be highly active against *S. aureus*, ponericin G1, was shown to have steady release after incorporation into an LbL film with no loss in antimicrobial activity while preventing *S. aureus* attachment.[46] These AMP-incorporated films also were found to be completely biocompatible with wound-healing cells.

The antibacterial activity of iron chelation and gallium (Ga^{3+}) therapies have attracted recent attention. These ions competitively bind biologically significant ions, lending an ability to destruct bacterial biofilms, particularly from the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* spp) group of pathogens. A recent mechanistic study indicated promising characteristics of the ability of Ga^{3+} to compete with the native ferric cation for binding acinetobactin (siderophore of *A. baumannii*).[47] Although early studies have suggested the potential development of Ga^{3+} resistance in *P. aeruginosa*, there remains much promise and interest in combining Ga^{3+}-based complexes with quorum quenchers as a "Trojan horse" approach compared with traditional monotherapy.[48,49] When incorporated into a hydrogel, Ga^{3+} has also demonstrated efficacy against *S. aureus* biofilms and enhanced wound healing by promoting collagen deposition and reducing inflammation.[50]

A unique and novel approach, molecular machines (MMs) are molecular motors that unidirectionally rotate in response to stimuli, resulting in a drilling-like rapid motion capable of propelling through a lipid bilayer typical of the bacterial cell wall membrane. MMs usually require UV light for activation, but recent studies describe multiple visible light-activated (405 nm) MMs that rapidly target bacteria as broad-spectrum antiinfectives without detectable resistance.[51] This technique is effective against planktonic bacterial cells and resistant phenotypes found in biofilms and persister cells. MMs cause loss of membrane potential by disrupting the cell membrane and leading to intracellular leakage but can also thus potentiate the action of traditional antibacterials by allowing access to intracellular targets if sublethal.

3.2. Gaps, Needs, and Next Steps

While infection prevention remains the ultimate goal, further research is essential to explore new preventative and treatment approaches. In addition, care must be taken to ensure that preventative treatments do not negatively affect soft tissues by hindering healing or causing local toxicity. Therefore, there is a clear need for innovative approaches that avoid drug resistance while offering low toxicity. New and emerging technologies such as antimicrobial peptides (AMPs), small molecules, and enzyme-

based techniques (eg, Dsp B and DNase I), which provide broad-spectrum activity, prevent resistance, minimize host tissue damage, and are cost-effective, are urgently needed in the field of BAL and OI. Given the relatively high frequency and serious consequences of infections, these strategies hold substantial potential. However, promising initial findings must be effectively translated through well-characterized and clinically relevant animal models to achieve clinical success.

The specific bioburden or antibiotic coverage in OI is unknown at this time, but most empiric or prophylactic antibiotic treatments, such as cephazolin, have Gram-positive activity and very little Gram-negative activity. The overall infection rate has stayed similar over the years while the Gram-negative rate (as percentage of infections) has increased. Contamination may always be present, and preventing Gram-positive microbes with antibiotics with activity against Gram-positives will drive organisms toward a Gram-negative population. Approaches to provide Gram-negative coverage exist, but concern remains about widespread prophylactic use of antibiotics for Gram-negatives because of their propensity for developing resistance. This represents a major research gap. An effective antimicrobial that does not cause host tissue damage while not causing resistance remains the elusive gold standard for basic science research studies. This overarching need for a drug, technology, or approach that does not drive drug resistance and offers low toxicity has driven much of the innovative and emerging work outlined in this section. Given that infections are fairly frequent and consequences can be profound in OI, the need for infection prevention and treatment strategies cannot be understated.

4. The Skin Penetration Aperture

Probably, the most eye-catching and medically most unique feature of osseointegration is the transcutaneous aperture region of the skin and the implant. Nearly every other surgery seeks skin closure on completion, although notable exceptions include temporary scenarios such as abscess drainage, certain situations of compartment release, or external fixators (which are generally considered the most related surgery to osseointegration). One other surgery that features both a permanent transcutaneous component and a bone-anchored device is cochlear implants. Urinary and intestinal reconstructions through the skin differ in that the epithelium heals, albeit to a different organ tissue. Given the uniqueness of BALRs protruding the skin and chronic unhealed skin surrounding a permanent metal foreign body anchored into bone, the directly relevant basic science research is limited. More research exists on bone-anchored hearing aids and oral osseointegration, although the biology is different and both bone-anchored hearing aids and oral osseointegration (dental implants) occur in more vascularized tissue with less infection complications.

4.1. Current Status

Most of the research on the penetration aperture surrounding the osseointegration implant has begun to shed light on the inflammatory processes at play.[52,53] In an early study using gross visual observation, light microscopy, and electron microscopy, Holgers et al[53] found that numerous immune system and inflammatory cells such as neutrophils and macrophages were present around all implants and increased cellular presence associated in situations where patients had clinically apparent inflammation around the aperture site. Very informatively, they did notice that macrophages could be found with bacteria engulfed, demonstrating the functional antibacterial activity of the immune cells. More evaluation of the specific cytokines present in the skin surrounding transcutaneous implants has been performed by others, also at the University of Gothenburg, on bone-anchored hearing aids.[52] They reported that through 12 weeks, multiple cytokines remained elevated. Studies of the inflammation within the skin and the skin–implant region are needed, which focus on longer term (perhaps 1 year or longer) to understand to what extent or in what ways the inflammatory milieu stabilizes.

The details of the skin attachment to implant surfaces also require additional attention. Holgers et al[53] also directly investigated this topic and reported that for a polished implant surface, hemidesmosomes facing the implant were never found, indicating that there was no evidence of true biological bond, regardless of the gross appearance that such a bond may have formed. While the authors of this consensus article are not aware of any true biologic bonding ever being substantiated between living tissue and foreign material, surface modifications seem to potentially stabilize the interface between skin and implants. Evaluating bone-anchored hearing aids, van Hoof et al[54] demonstrated that the surface of bare titanium implants is covered by unstructured skin elements, protein, and lipids, whereas the areas covered by hydroxyapatite were more uniformly and completely covered by organized keratinocytes and collagen fibers, with no appearance of nonviable corneocytes or cellular debris. Again, antimicrobial phagocytosis was observed, suggesting that appropriate immune activity remained. However, this apparently improved biologic environment may not provide better outcomes through a period of 1 year.[55] Surface modification for oral osseointegration implants also affects early gingival stability.[56] Certainly, the skin and gingival stability and forces for bone-anchored hearing aids and oral osseointegration versus limbs are very different and not assumed to be extrapolatable. In the scenario of external fixation, hydroxyapatite coating of pins tends to prevent pin loosening.[57] Hydroxyapatite and perhaps other surface modifications merit further exploration specifically regarding the skin interface.[58]

Many research efforts are now focused on methods to optimize this soft-tissue layer. Engineering epithelial attachments to the implant can allow reduction in soft-tissue breakdown and inflammation, which in turn may decrease risk of infection. Several studies have shown that introduction of a porous surface can reduce exposure to sheer stresses of epithelial cells.[15,59] Some of the same methods used to the implant surface directed at enhancing osseointegration, and/or prevention of bacterial adhesion can also promote activation and adhesion of epithelial cells to strengthen the skin interface. Extracellular matrix (ECM) peptides, such as fibronectin and E-cadherin, have been shown to promote adhesion of keratinocytes to titanium surfaces.[60,61] One study demonstrated that by coating titanium implants with keratin, one of the abundant proteins found in a naturally occurring infection-free skin interface of the fingernail–skin junction, enhanced fibroblast and keratinocyte adhesion as well as promoted a stable phenotype.[62] Attempts to improve dermal integration are critical but have so far failed overall in both preclinical and clinical translation. Thus, the current state of the art and clinical practice of all OI devices presently exhibit a smooth, polished abutment interface to decrease skin irritation, deter bacterial attachment, and improve hygiene of the skin penetration site.

4.2. Gaps, Needs, and Next Steps

The gaps and needs regarding the skin penetration aperture for BALR likely center around understanding to what extent skin adherence to the implant is beneficial. Historical research by Hall et al suggested that skin adherence to an implant seemed to pull it out of the bone,[63] although that was using implants substantially inferior to those available today. Skin matures and migrates through its approximately 40–60 days of life span[64] from basal cell to exfoliated corneocyte. Multiple questions need to be understood to confidently produce implants or use techniques for large numbers of patients. Does surface modification to improve skin adhesion result in skin injury as migration pulls the skin along the implant? Does this negatively affect the inflammatory milieu at the skin penetration aperture site? Does the skin adhesion overpower the bone–implant interdigitation? Does the surface adhesion persist after multiple generations of skin over months to years interacting with the surface? Might such a tight seal possibly increase the rate of deep infection by sealing in bacteria rather than allowing drainage, in other words promoting an abscess instead of allowing a sinus? Admittedly, the relevance of some of these questions likely will be influenced by whether potential technique variations (such as skin graft–type skin stabilization techniques) or better understanding of infection prevention (whether the bone itself rather than soft tissue actually prevents bacterial ingress) reveals such skin implant needs to be necessary or moot.

5. Noninfectious Inflammatory Loosening

5.1. Current Status

Aseptic loosening is a term often used to refer to implants that have lost stable fixation within the bone in the absence of evidence for infection. Aseptic loosening, or noninfectious inflammatory loosening, has been reported in osseointegration literature, but it has remained relatively infrequent and has been further minimized by improvements in surgical technique and implant technology.[65–68] While many patients do not show active signs of infection, the loosening typically progresses from the skin-penetrating abutment (SPA) upward along the implant where there is no wear or bearing surface to create another source of inflammation. This may be due to ascending bacterial inflammatory process or colonization. This presents an area for further investigation to better understand the underlying mechanism.

5.2. Gaps, Needs, and Next Steps

Research focused on evaluating the extent to which bone–implant integration can resist bacterial ingress, compared with skin, muscle, or other mechanisms. Such findings would help to inform other domains of research that have the potential to contribute infection prevention (eg, achieving skin–implant stability). One potential goal for future studies could be developing adjuvant therapies to enhance the skin barrier as the primary defense against bacterial ingress. In addition, the concept of "non-infectious inflammatory loosening" also warrants further research and improved characterization. There remains ongoing debate about whether these cases are truly aseptic and non-infectious or whether current limitations of bacterial culturing and microbiology techniques fail to detect an underlying infection. Similarly, the mechanism by which noninfectious loosening occurs represents a gap in knowledge. The presence of bacteria is known to cause an immune cell response and a bone cell response (eg, osteoblast release of RANK-L that causes osteoclast differentiation and bone adsorption). What remains to be understood is the cause of this loosening, and whether it is truly aseptic and the result of another inflammatory or biological factor, or loosening from a colonization that does not present any signs of infection.

6. Other Topics

Other basic science research topics, such as those related to bone integrity, represent areas of potential interest, but ones the authors do not view as a basic science priority at the time of this publication. Periprosthetic fractures are a clinically significant outcome, but the BALR implant itself is likely not a causative factor of fracture since the fractures do not occur without a true traumatic situation.[69–71] Indeed, metabolic bone research (including osteoporosis, aging, and those caused by cancer treatment [ie, hormone-induced or radiation-induced bone changes]), is currently lacking for BALR care. However, improvements and progression in therapies will likely derive and benefit from other non-BALR research areas. It is important to establish how existing therapies can be applied to the OI population, such as DEXA scans, bone density tests, bisphosphonates, and denosumab. For example, understanding the response of osteoporotic bone to BALR implants in the setting of bisphosphonates may be more readily investigated in the clinical setting as many amputated patients have low density or osteoporotic bone from extended disuse.[72] From a basic science perspective, bisphosphonates are already well characterized to improve bone density by permanently deactivating osteoclasts but at the expense of impaired remodeling, a critical process of implant–bone integration. Physical rehabilitation (the focus of another topic article in this Special Issue to which the authors here defer) and the improved mobility that often accompanies BALR patients will also confer meaningful impacts on bone integrity and quality, an outcome that is often already reduced by virtue of being an amputee.[72] Although important and crucial to the overall success of OI implants, this is another outcome that is difficult, if not completely unreliable, to measure with basic science studies.

7. Conclusions and Research Recommendations

As an emerging field, the basic science of OI benefits from advances in related fields such as orthopaedic and implant infections. However, its emergent status has left many substantial gaps that lag behind clinical needs. As more BALR procedures are performed, the growing patient population will require lifelong improvements in care. The gaps outlined in this review underscore the critical importance of advancing basic science research. While early studies may draw from related fields, future research must prioritize OI-specific models and contexts. Standard approaches should accurately reflect key clinical characteristics, such as the skin penetration aperture and implant surface features to effectively address the unique challenges of OI. Furthermore, there is an evident need for well-established preclinical animal models, as clinical trials often rely on such preclinical studies for safety, efficacy, and mechanistic data. Addressing these challenges will require collaborative efforts between surgeons, physician scientists, research scientists, and engineers.

It is important to emphasize that, although small in size, this clinical population is often young and these challenges endure throughout the entire patient's lifetime. There are not many mature and established solutions or therapies for these problems,

and lack of a representative and predictive (of clinical outcome) preclinical model is a big hurdle that will prevent translation to clinical use. Moreover, the relatively small number of patients and the profound impact of these complications on their long-term outcomes should necessitate the investment in basic science studies and development and validation of animal models a top priority.

In summary, the authors provide consensus recommendations on the critical need for infection-focused basic science research made possible, in part, by the development of animal models as a top priority. The current state of infection prevention and treatment research studies outlined in this article highlights where further resources are needed. Important clinical applications of all infection approaches must be considered, including surveillance of the impact of hygiene care of the skin penetration aperture site and the role of bone integrity and osseointegration in infection. Because of the unique nuances of OI/BALR and the inability of other OI-related technologies or protocols to advance in the face of an infection challenge, we propose that any infection topic should be considered to benefit considerably from basic science understanding.

Appendix 1. Collaborators

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