Emerging Orthobiologic Techniques and the Future

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KEYWORDS

• Orthobiologics • Future • Stem cells • PRP

KEY POINTS

- Ideal development of orthobiologics products should follow a developmental pyramid of evidence to prove safety and efficacy before widespread clinical application.
- Understanding regulatory classification and development is a key to translation of orthobiologics from animal study to clinical practice.
- There are emerging orthobiologics technologies that have followed the developmental pyramid; these will likely sustain the tests of time.

INTRODUCTION

Orthopedic sports medicine has advanced tremendously in the last 30 years, with most of the innovation surrounding the arthroscope and associated techniques. Advancement has been optimized when a pyramid of development has been pursued, and with Orthopedics, this has traditionally involved quantitative anatomy as the base, followed by biomechanical study, clinical application, revision with outcome data, and evidence-based clinical application at the pinnacle. The use of a developmental model has been termed translational biomechanics and illustrated as a pyramid. It has become clear that the next 30 years of advancements within sports medicine will involve the advancement of orthobiologics. A recent lesson is clear with one of the first orthobiologics, platelet-rich plasma (PRP). Clinical application without development leads to confusion among clinicians, industry, and patients about mechanism of action, safety, efficacy, and overall value. The future of orthobiologics lies in using a similar pyramid of development as translational biomechanics, with preclinical bench-top and animal studies at the base, followed by pilot clinical trials, controlled

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comparative clinical trials, multicenter study, and clinical application at the pinnacle (Fig. 1).[1] Although daunting, expensive, and time consuming, developing this pyramid, sometimes called translational medicine, is the future of orthobiologics. Technologies that short-circuit the process will likely fade into history.

Clinical application of orthobiologics without development produces confusion and stagnates progress. Progress currently faces a delicate balance with providers and patients sprinting toward application of emerging technologies on one side and the marathon of technology development through translational medicine on the other side. Weighing the balance is the orthopedic community, the public, and government regulatory bodies. Scientists and clinicians must understand and embrace yet challenge the development pathway, to refine it, because the next steps of translation require patient care and clinician participation. A potential pitfall, industry may at times present biased interpretations of regulation and the developmental process to clinicians and patients, but ultimately patients as well as regulatory bodies expect physicians to understand the regulation of medical treatments that they offer. For this reason, this article reviews the principles of biologic product development and the regulation surrounding the development and discusses emerging technologies that are walking the path of development.

PRINCIPLES OF DEVELOPMENT FOR ORTHOBIOLOGICS

The foundational principles of development are mechanism of action, safety, and efficacy. Safety involves ensuring that in the course of administering a product in an appropriate fashion the product does not cause harm, injury, or loss by the recipient in a direct or indirect manner. For orthobiologics, safety often involves avoiding the possible introduction, spread, and/or transmission of infectious disease as well as ensuring that treatments do not cause undue adverse events. Adverse event concerns include the possibilities of immune reactions to biologic treatments, infections, the potential for neoplasms, and/or increasing the likelihood of a venous thromboembolic event. Efficacy generally involves the power of a treatment to produce a claimed effect. As new biologic treatments are emerging, the orthopedic community should consider that the due diligence of mechanism of action, safety, and efficacy should

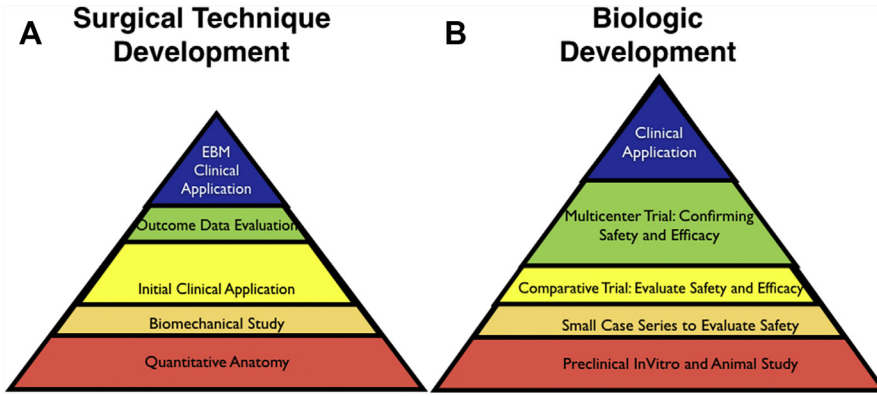

Fig. 1. (*A*) Translational biomechanics has been the developmental model of evidence-based orthopedic technique progress. (*B*) The future of orthobiologics will follow a similar developmental model. EBM, evidence based medicine. (*Courtesy of* Dr Adam Anz, Gulf Breeze, FL; with permission.)

precede marketing and/or making claims regarding biologic treatments. The Food and Drug Administration (FDA) was founded on the principle of ensuring that products are safe and effective before use as medical treatments. The FDA has been given the responsibility to protect the public from unproven treatments, and since 2008, the reach of the FDA has extended into both industry and the clinical practice of medicine regarding orthobiologics.

Monitoring and regulation of orthobiologics is a double-edged sword, important for patient safety and proof of worth on one side, but seemingly stifling to progress on the other. Loose regulation encourages clinical experimentation, but raises concerns for patient safety, and does not force products to prove their value before clinicians set prices, market, and use them for patient treatments. Although rigid regulation stifles progress, it ensures patient safety and forces technologies to prove themselves through a developmental process. The latter requires a significant investment of time and money, but produces clear indications and evidence for care.

FOOD AND DRUG ADMINISTRATION CLASSIFICATION OF ORTHOBIOLOGICS

Understanding the FDA's mechanisms is important for clinicians seeking to use orthobiologics and/or participate in the developmental process. To the FDA, most biological products are a subset of drugs,[2] and "biological" refers to those medical products that are derived from living material, as opposed to chemically synthesized.[2] The FDA does not consider everything that clinicians consider orthobiologics as biological products. The FDA applies the *Federal Food, Drug, and Cosmetic Act* for the monitoring and regulation of many orthobiologics especially those involving cells. The FDA derives its authority to regulate biologic products from the Public Health Service Act (PHSA), a federal law enacted in 1944 that outlines the federal government's duties to protect the health of the public. Section 351 of the PHSA (PHSA 351) addresses biological products defined as "virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, applicable to the prevention, treatment, or cure of a disease or condition of human beings."[2] PHSA 351 established the authority for the FDA's oversight in the development of these products. Section 361 of the PHSA (PHSA 361) granted the FDA the authority to prevent the spread of communicable diseases.

As biologics have emerged in medicine, the FDA has developed layered regulations, based on perceived risk to the United States Public, which set the mechanisms of control and oversight established in PHSA 351 and PHSA 361. These regulations are set forth in the Code of Federal Regulations. The Code of Federal Regulations is a document produced yearly that depicts the rules published in the Federal Register. These rules are established by the Executive departments and other agencies within the Federal Government. This document depicts the policies of the FDA and contains specific instructions to manufacturers, health care providers, and sponsors in the development/manufacture of products. Title 21 specifically focuses on the rules of the FDA. Part 1271 of Title 21 (21CFR 1271) is titled: Human Cells, Tissues, and Cellular and Tissue-based Products, or HCT/Ps for short, and addresses "articles containing or consisting of human cells or tissues that are intended for implantation transplantation, infusion, or transfer into a human recipient."

21CFR 1271 states that an HCT/P is regulated solely under 361 of the PHSA and must be manufactured to meet the requirements of 21 CFR 1271 alone if it meets 4 criteria: (1) the HCT/P is minimally manipulated; (2) the HCT/P is intended for homologous use only; (3) the manufacture of the HCT/P does not involve the combination of the cells or tissues with another article, except for water, crystalloids, or a sterilizing,

preserving, or storage agent, provided that the addition of water, crystalloids, or the sterilizing, preserving, or storage agent does not raise new clinical safety concerns with respect to the HCT/P; (4) either the HCT/P does not have a systemic effect and is not dependent upon the metabolic activity of living cells for his primary function or if the HCT/P does have a systemic effect or is dependent upon the metabolic activity of living cells for its primary function, it is autologous or allogenic in a first-degree or second-degree blood relative (**Box 1**).[3] HCT/Ps that meet these 4 criteria are often termed "361 products." Whereas these HCT/Ps are not subject to premarket FDA review requirements, 1271 does set forth clear requirements within 6 domains: (1) registration and listing with the FDA, (2) donor screening and testing, (3) current good tissue practices, (4) labeling, (5) adverse-event reporting, and (6) inspection and enforcement. Certain exemptions for the requirements set in 1271 exist for some HCT/Ps, which are harvested, processed, and reimplanted in the same surgical procedure and are exempt from the requirements of CFR 1271; however, they are not exempt from overall regulation under PHSA 361 and/or 351. Guidance documents suggest that examples for exemption include veins harvested for coronary artery bypass grafting and cranial tissue harvested and stored for reimplantation at a later date.

HCT/Ps that do not meet criteria described in CFR 1271 are regulated as a drug under section 201(g) of the Federal Food, Drug, and Cosmetic Act, a device, and/or a biological product as outlined in PHSA 351 of the PHS Act. These products, often termed "351 products" are subject to premarket and postmarket development requirements and FDA approval before they can be marketed. In addition, their manufacture must comply with both current good tissue practices and current good manufacturing practices. Development requirements involve a series of steps often called the "351 pathway" and start with preclinical laboratory and animal testing to show that investigational use would be safe in humans. Before initiating clinical studies

Box 1
In order for an orthobiologic to be considered low risk by the Food and Drug Administration, it must meet 4 criteria

1. Minimal manipulation:
   The HCT/P is minimally manipulated
2. Homologous use:
   The HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent
3. None combination product:
   The manufacture of the HCT/P does not involve the combination of the cells or tissues with another article, except for water, crystalloids, or a sterilizing, preserving, or storage agent, provided that the addition of water, crystalloids, or the sterilizing, preserving, or storage agent does not raise new clinical safety concerns with respect to the HCT/P
4. None systemic effect or autologous:
   Either:
   i. The HCT/P does not have a systemic effect and is not dependent upon the metabolic activity of living cells for its primary function
   ii. The HCT/P has a systemic effect or is dependent upon the metabolic activity of living cells for its primary function, and
      a. Is for autologous use;
      b. Is for allogeneic use in a first-degree or second-degree blood relative; or
      c. Is for reproductive use.

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in humans, an investigational New Drug Application (IND) must be in place as described in 21 CFR 312. Subsequent clinical trials prove safety and efficacy in a phased fashion, most often first involving small pilot human study followed by large multicenter pivotal study. The FDA has illustrated recent flexibility in pathway design dependent on the product under development requiring only pilot and pivotal studies in some instances. Results demonstrating safety and efficacy for an indication are submitted to the FDA as part of a biologics license application (BLA). Approval of a BLA is required before marketing or administration of the product in clinical practice. Several milestones are recognized through the process, and the sponsor communicates with the FDA at multiple time points to guide the process.

Although orthopedists may consider it a surgical procedure, the FDA considers the process of taking tissue from an individual, processing the tissue, and replacing the tissue as the manufacture of a product.[4] It is important to highlight that, although PHSA 351 specifically states that blood or blood components are biologic products, the FDA has expressly stated that whole blood, blood components, and minimally manipulated bone marrow for homologous use are not considered HCT/Ps in guidance documents and has no precedent of regulating the application of these products by clinicians.[4] However, through untitled letters, warning letters, statements of the tissue reference group, and recently finalized guidance documents, the FDA has set precedent for autologous products produced from adipose tissue, allograft products derived from human placenta, allograft cell products, autologous cultured cell products, and autologous hematopoietic stem cells, suggesting that they regard these as 351 products. Although clinicians and industry may take liberty with interpretation in some instances citing a same surgical procedure exemption, the FDA has recently made clear statements removing ambiguity.[4,5]

In November 2017, the FDA released 2 guidance documents to aid clinicians and industry pertinent to orthobiologics. One document sought to clarify homologous use and minimal manipulation and used specific examples regarding adipose and placenta/amnion-derived products. This document clarified requirements of developmental process before clinical application.[4] The second document clarified the FDA's intent behind 21CFR1271.15(b), an exemption clause related to the setting of a surgical procedure. This document used the example of adipose tissue and stated that an establishment that harvests adipose tissue, processes the tissue by enzymatic or mechanical processes, and injects the product would not qualify for the exception.[5] Although the guidance documents are subject for interpretation, a subsequent publication in the *New England Journal of Medicine* authored by the director of the FDA's Center for Biologics Evaluation and Research and commissioner of the FDA clarifies the FDA's intent and interpretations. The FDA's goal is to facilitate innovation but ensure that emerging techniques prove they are safe and effective.[6]

EMERGING TECHNOLOGIES BUILDING A DEVELOPMENTAL PYRAMID

Although at times clinical application has outpaced development, there are many technologies that have leveraged the process to build a pyramid of developmental evidence. Review of a sample can help the clinician understand progress and gain a vision of the future of orthobiologics. Emerging techniques to treat osteoarthritis (OA), augment anterior cruciate ligament (ACL) repair/reconstruction, and improve cartilage repair have decades of developmental progress and represent the tip of the spear as well as technologies of the future.

Osteoarthritis

The first technology with a pyramid of development is point of care blood products, that is, PRP, for the indication of OA. At the top of this pyramid are recent systematic reviews and meta-analyses of comparative clinical trials that are clarifying a consensus that leukocyte-poor PRP is an effective intra-articular treatment for knee OA. The base of the pyramid began with animal and bench-top studies.

In bench-top studies, PRP has a clear mechanism of action to improve the catabolic and inflammatory environment of OA. Van Buul and colleagues[7] investigated the effects of PRP releasate upon cartilage cells that had been exposed to interleukin-1 (IL-1) beta, one of the most caustic inflammatory proteins within the osteoarthritic joint. They found that PRP releasate diminished multiple inflammatory effects of IL-1 on chondrocytes in culture, including reducing the activation of nuclear factor kappa B, a nuclear factor that upon activation translocates to the nucleus of cells and activates genes involved in apoptosis, inflammation, and other immune responses. Additional bench-top studies have shown that PRP stimulates proliferation of chondrocytes in culture,[8] decreases production of matrix metalloproteinases by synovial cells, and decreased inflammatory gene expression in an OA model.[9]

Preclinical animal studies have reflected bench-top progress. Saito and colleagues[10] investigated the effects of PRP on the progression of OA in a rabbit model. PRP in gelatin hydrogel microspheres was administered twice intra-articularly 4 weeks after ACL transection, a method to create a model of OA. At 10 weeks after the transection, cartilage samples illustrated superior histologic and morphologic scores in the PRP group, and the PRP group expressed significantly more proteoglycan messenger RNA. In a similar model, Yin and colleagues[11] evaluated the effects of a 3-PRP-injection regimen, comparing leukocyte-rich and leukocyte-poor PRP. Both the leukocyte-rich and the leukocyte-poor injections achieved better morphologic and histologic scores compared with a control, but the leukocyte-poor group had the best scores as well as reduced concentrations of inflammatory proteins. Similar study in mice involved an OA model created with intra-articular injection of a collagenase. A 3-injection series of PRP releasate reduced pain and synovial thickness when compared with a 3-injection series of saline.[12]

Clinical trials in humans have established safety and efficacy beginning with early small case series, followed by larger well-designed comparative cohort and randomized trials, and completing with systematic reviews of the literature. The orthopedic community is moving toward a consensus that intra-articular leukocyte-poor PRP is a safe and effective treatment for OA. A recent systematic review of the literature found 29 well-designed studies including 26 evaluating knee OA and 3 evaluating hip OA. The current status includes 9 prospective randomized controlled trials (RCTs), 8 knee and 1 hip, 4 prospective comparative studies, 14 case series, and 2 retrospective comparative studies. As a comparative group, hyaluronic acid (HA) was used as a control in 11 studies (7 RCTs, 2 prospective comparative studies, and 2 retrospective cohort). Only 2 RCTs, one for knee and one for hip, did not report significant superiority of PRP compared with the control group; in both of these studies, HA was used as a control. Nine out of 11 HA controlled studies showed significant better results in the PRP groups.[13]

One particular leukocyte-poor preparation deserves attention because it is progressing through clinical trials with the FDA, which will validate the technology to the entire orthopedic community, both national and international, as well as to payers. One company has developed a disposable that creates leukocyte-poor PRP, which they have branded as autologous conditioned plasma (ACP) and which is seeking an FDA

approval. Clinical evaluation has 2 studies to highlight. Cerza and colleagues[14] compared the clinical response of HA to ACP in 2 groups of patients affected by knee OA. One hundred twenty patients were randomized to 2 groups: 60 patients received 4 weekly intra-articular injections of HA and 60 patients received 4 weekly injections of ACP. A significant effect was evident in the ACP group shortly after the final injection, and the effect improved up to 24 weeks (**Fig. 2**A).[14] Clinical outcomes were better than the results obtained with the HA based on Western Ontario and McMaster (WOMAC) score. ACP showed a significantly better clinical outcome than HA.[14] Development continued with a goal of FDA approval for knee OA beginning with an FDA-observed pilot study geared toward safety. One hundred fourteen patients were screened to yield 30 patients, randomized to 2 groups. A series of 3 weekly injections was studied, with saline as a control. WOMAC score served as the primary efficacy outcome measure, and patients were followed for 1 year. No adverse events were reported, and at conclusion, WOMAC scores for the ACP subjects had improved by 78% from baseline, whereas scores for the placebo group had improved by only 7% (**Fig. 2**B).[15]

Currently ACP is under multicenter evaluation for pivotal study. If the results of the pivotal study reflect the pilot data, an FDA approval can be expected. An FDA approval is the first step in obtaining CMS coverage and will provide leverage for the orthopedic community to seek reimbursement from private insurance companies. Because a developmental pyramid has been built, PRP is a technology that with continue to evolve, develop, and remain in the future, rather than a technology that will fade.

ANTERIOR CRUCIATE LIGAMENT REPAIR/RECONSTRUCTION

Another technology with developmental progress is the biologic augmentation of ACL repair and reconstruction. Martha Murray, MD, and her team at Boston Children's Hospital[16] have laid much of the instrumental preclinical groundwork, and progress to clinical

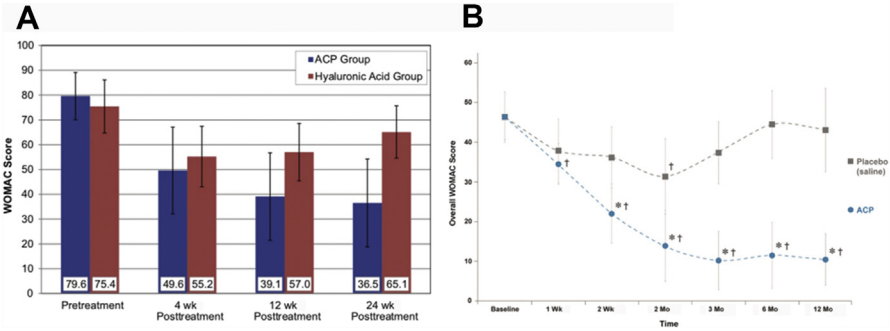

Fig. 2. (*A*) Results from an RCT in 120 subjects comparing mean WOMAC scores for ACP and HA groups. (*B*) Results of an FDA observed RCT in 30 subjects comparing a 3-weekly injection series of ACP to saline. Overall Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores versus time for the autologous conditioned plasma (ACP) and saline placebo treatment groups. * Significant difference from saline placebo ($P < .05$); † Significant difference from baseline within each respective group ($P < .05$). (*From* [*A*] Cerza F, Carnì S, Carcangiu A, et al. Comparison between hyaluronic acid and platelet-rich plasma, intra-articular infiltration in the treatment of gonarthrosis. Am J Sports Med 2012;40(12):2822–7, with permission; and [*B*] Smith PA. Intra-articular autologous conditioned plasma injections provide safe and efficacious treatment for knee osteoarthritis: an FDA-sanctioned, randomized, double-blind, placebo-controlled clinical trial. Am J Sports Med 2016;44(4):884–91, with permission.)

trials has been achieved. A central premise has emerged that a key to ACL healing and remodeling is providing an early, stable scaffold for the invasion of reparative cells. A key moment is a canine study evaluating a type-I collagen sponge loaded with a PRP hydrogel. A central defect was created in the ACL of a group of canines. Defect healing was evaluated with and without biologic enhancement. Repair tissue evaluated at 3 and 6 weeks showed better fill in the scaffold group at both 3 and 6 weeks. When tested biomechanically, the biologic scaffold group had 40% increased strength.[17]

Through preclinical research, development has continued clarifying the ideal initial repair construct, the best biologic addition, and appropriate metrics to evaluate healing. Regarding repair construct, Murray[18] found that bone to bone stabilization, with an internal splint, outperformed repair alone in a porcine model, improving the structural properties of healing tissue (**Fig. 3**). This concept has also been evaluated in a cadaver model of ACL reconstruction and found to significantly reduce elongation

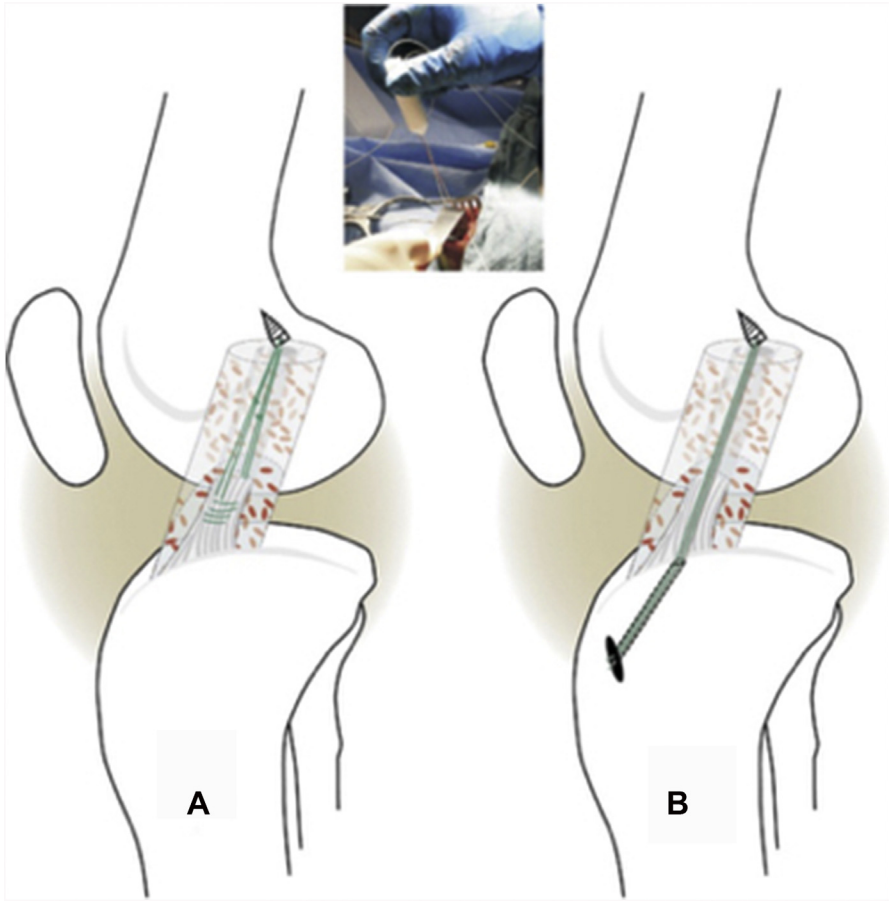

Fig. 3. A porcine animal model comparing ACL repair securing to the tibial stump alone (*A*) with repair including sutures through a tibial bone tunnel (*B*) found improved yield load and stiffness with the bone tunnel group but similar laxity in both groups. (*From* Murray MM, Magarian E, Zurakowski D, et al. Bone-to-bone fixation enhances functional healing of the porcine anterior cruciate ligament using a collagen-platelet composite. Arthroscopy 2010;26(9):S49–57. Figure 1; with permission.)

when cyclically loaded and produced a higher ultimate failure load without stress-shielding the graft.[19]

Different biologic enhancements have also been studied in preclinical development. In a minipig, bone-tendon-bone ACL reconstruction model, extracellular matrix (ECM) scaffolds were loaded with different PRP preparations. Although loading the scaffold with a PRP preparation similar to whole blood produced a biomechanically superior construct, loading a scaffold with increased platelet concentration PRP (3-fold and 5-fold) did not (**Fig. 4**).[20] Evaluating cultured cells in an ACL repair model, 3 methods of bioenhanced repair, an ECM matrix loaded with whole blood, an ECM matrix loaded with cells cultured from the fat pad, and an ECM matrix loaded with cells cultured from the buffy coat of whole blood were compared. After 15 weeks of healing, similar biomechanic and histologic properties were found between all groups, leading investigators to determine that whole blood is a sufficient biologic to augment repair and reconstruction.[21] Similar study has evaluated the biomechanic properties of tendon grafts loaded with bone marrow–derived cultured stem cells in a porcine model. Loading allograft tendons with cells and subjecting them to dynamic mechanical stimuli significantly enhanced matrix synthesis and ultimate tensile load after implantation.[22]

After refining bridge enhanced repair, Vavken and colleagues[23] compared repair to reconstruction in a porcine model. The repair group demonstrated no biomechanic difference and had less evidence of macroscopic cartilage damage when compared with reconstruction. Although biomechanic, morphologic, and histologic studies are sufficient outcome measures for preclinical animal studies, translation to human clinical trials requires noninvasive outcome measures. Biercevicz and colleagues[24,25]

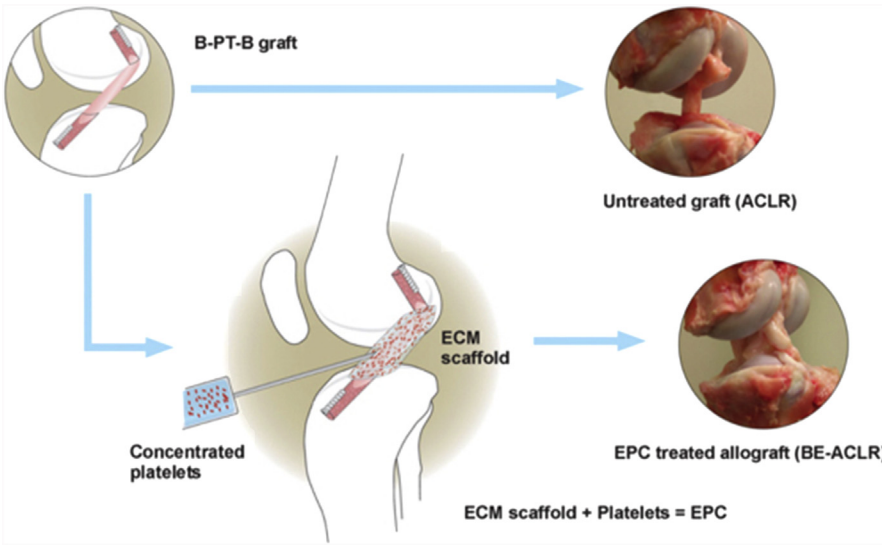

Fig. 4. Murray and colleagues evaluated standard ACL reconstruction to a bio-enhanced ACL reconstruction with differing concentrations of PRP. The scaffold is loaded with plasma containing a platelet concentration equal to blood performed superiorly when evaluated biomechanically. ACLR, anterior cruciate ligament reconstruction; BE-ACLR, bridge enhanced anterior cruciate ligament reconstruction; B-PT-B, bone patellar tendon bone. (*From* Fleming BC, Proffen BL, Vavken P, et al. Increased platelet concentration does not improve functional graft healing in bio-enhanced ACL reconstruction. Knee Surg Sports Traumatol Arthrosc 2015;23(4):1161–70; with permission.)

used 2 separate studies performed at Brown University to demonstrate that volume measurements and grayscale values from high-resolution T2 images were predictive of structural properties of ACL healing in a porcine model.

Murray began clinical study after an Investigational Device Exemption was obtained from the FDA.[26] A prospective cohort study compared bridge-enhanced ACL repair and standard autograft hamstring reconstruction. At 3-month follow up, neither group had any joint infections or signs of significant inflammation, and upon Lachman examination, the bridge-enhanced ACL repair had 8 International Knee Documentation Committee (IKDC) grade A examinations and 2 IKDC grade B examinations, whereas the ACL reconstruction group had 10 IKDC A examinations. MRIs from all patients demonstrated a continuous ACL or graft. Hamstring strength at 3 months was significantly better in the repair group. Longer follow-up will help clarify clinical performance. A similar, prospective randomized study is underway at the investigator's institution comparing standard ACL reconstruction to reconstruction augmented with a collagen matrix wrap seeded with bone marrow aspirate in both hamstring and patellar tendon ACL reconstructions (**Fig. 5**).[27]

Cartilage Repair

The largest developmental pyramid to date involves cartilage repair. Emerging techniques have been entrenched in development for decades, beginning with bench-top research, continuing with preclinical animal studies, and taking strides in the last decade through well-designed clinical trials. A clear pyramid of development has been built and guides emerging clinical application.

Although the earliest work on stem cells is attributed to Alexander Maximow at the University of Chicago in the 1920s,[28] foundational work applying stem cells to cartilage repair started on the bench top of Arnold Caplan in the late 1970s.[29] Through continued bench top and animal work all over the world, the mechanisms and logistics are becoming clear. Stem cells can be induced into cartilage cells, with work starting with bone marrow–derived cultured cells.[30] Cells from other tissue sources have also shown potential to differentiate to cartilage, including cells derived from adipose, periosteum, synovium, and muscle.[30–34] Because multiple cell sources have proven productive in bench-top study, the logistics around processing and application in light of regulatory/developmental requirements has guided further translation.

Bench-top work progressed to animal study in the early 1990s. In a rabbit model, implanted bone marrow–cultured MSCs on a collagen gel differentiated into chondrocytes by the second week after implantation, and tissue had organized into cartilage tissue with development of a subchondral bone plate by the 24th week.[35] Similar studies have followed with adipose,[36,37] synovium,[38,39] and periosteum.[40] With considerations of developmental and regulatory hurdles, researchers have also studied bone marrow aspirate concentrate as an adjunct to cartilage repair procedures. Bone marrow aspirate concentrate implanted at the time of a marrow stimulation procedure as a single implantation or series of injections after a marrow stimulation procedure have been shown to improve cartilage repair in an equine and caprine model.[41,42]

In addition to implantation of cells within a scaffold, another tested concept is that stem cells injected into a local environment, that is, a joint, have the potential to home (or localize) to an area of injury and participate in cartilage healing. Lee and colleagues[43] investigated this concept in a mini-pig. After the creation of a cartilage defect, one group received an intra-articular injection of stem cells cultured from bone marrow (BMSC) (average 7 million cells) suspended in HA followed by 2 additional weekly HA injections; another group received 3 weekly HA injections, and a third group received 3 weekly saline injections. Although both the HA and the BMSC groups were superior to saline, the

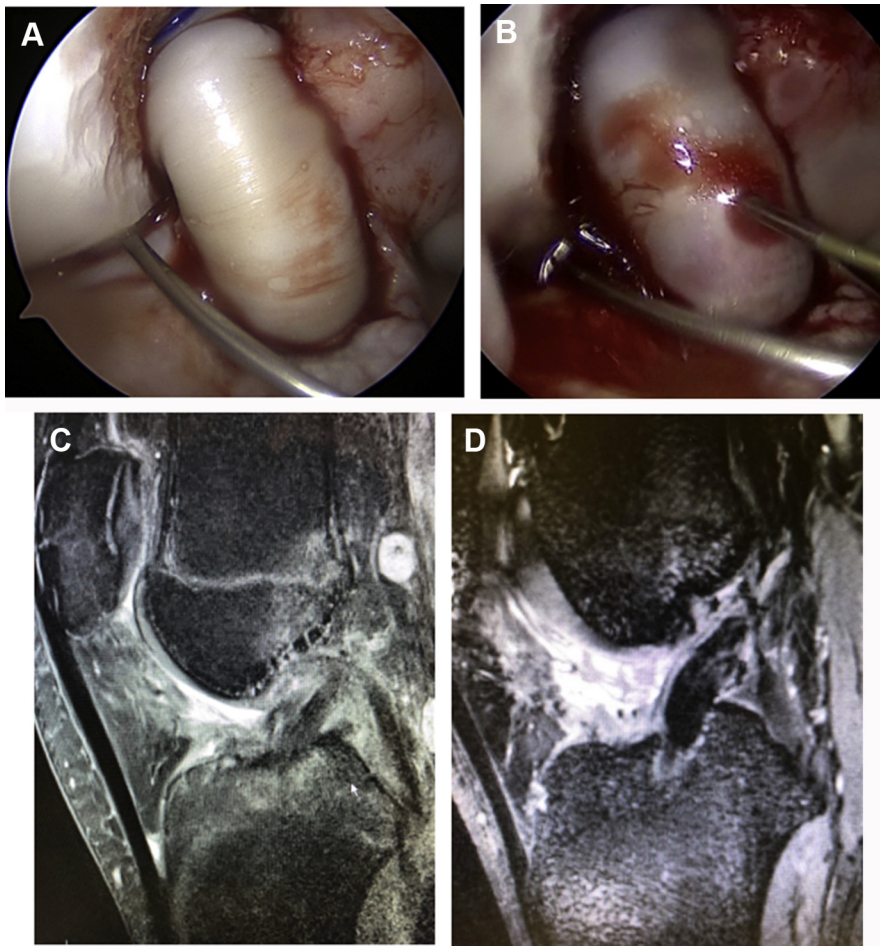

Fig. 5. Current RCT underway at the authors' institution comparing an ACL autograft wrapped with collagen matrix to a control group. Wrapped grafts are implanted (*A*) and then injected with bone marrow aspirate concentrate (*B*). MRI image of grafts in a control group (*C*) will be compared with intervention (*D*) at 3 months, 6 months, 12 months, and 24 months. (Original images from Steve Jordan, Adam Anz, James Andrews, unpublished data, 2018; and *Courtesy of* Dr Adam Anz, Gulf Breeze, FL; with permission.)

BMSC group stood out upon histologic and morphologic evaluation. The cells were also labeled with carboxyfluorescein, and upon histologic examination the labeled cells had homed to and integrated into the repair tissue. A similar study involving intra-articular injection of stem cells instead of direct open implantation has been performed with the same conclusions drawn in a meniscus injury model involving cultured synovial derived stem cells[44] and a large-animal model involving BMSC.[45]

Standing on a broad base of preclinical evidence, human studies have emerged and continue to emerge in 3 phases: case report/series design, comparative treatment study, and randomized controlled study. Recent systematic review found 60 clinical studies, including 9 case reports, 31 case series, 13 comparative trials, and 7 randomized controlled studies.[13] Stem cell treatments for cartilage repair are emerging as a safe and effective treatment, yet further well-designed comparative study is needed.

One stem cell technology that has been developing through FDA trials involves mobilized peripheral blood stem cells (PBSC). This technology follows the footsteps of the hematology oncology profession's development of the harvest of stem cells for bone marrow transplant. Although originally bone marrow transplant involved bone marrow aspiration harvest, the profession developed harvest via pharmaceutical mobilization followed by venous harvest with apheresis. Pharmaceutical mobilization stimulates an upregulation of production of cells in the bone marrow and release of these cells to the peripheral circulation. Apheresis harvest involves a machine that uses centrifugation, optics, and continuous venous access for a period of 1 to 4 hours to collect PBSC. For example, with orthopedic indications in mind, a 140-mL harvest contains on average 140 million $CD34^+$ cells, a quality control marker used to monitor stem cell numbers for bone marrow transplant. The harvest can be aliquoted and stored for serial/multiple injections[46] (**Fig. 6**).[47] These cell sources have established safety data involving large registries and cell characterization study, suggesting more immaturity than BMSC and functional properties similar to embryonal stem cells.[48,49] One striking advantage of this cell source is the ability to harvest millions of cells at one time point, which can be aliquoted and stored for serial injections throughout the maturation phase of cartilage healing. In addition, this technology leverages established techniques developed for bone marrow transplant and the body's potential to create stem cells to produce hundreds of millions of cells, without cell culture.

Developmental work applying PBSC to cartilage repair has emerged from a group in Malaysia. Lee and colleagues[43] first reported a case series involving arthroscopic marrow stimulation followed by multiple postoperative intra-articular injections in 5 patients, with safety data and histology suggesting good cartilage repair tissue. The case series was followed by an RCT comparing arthroscopic marrow stimulation followed by 8 postoperative PBSC intra-articular injections over the course of 6 months to arthroscopic marrow stimulation followed by 8 postoperative HA intra-articular injections. At 2 years, histology and MRI results favored the treatment group, and the

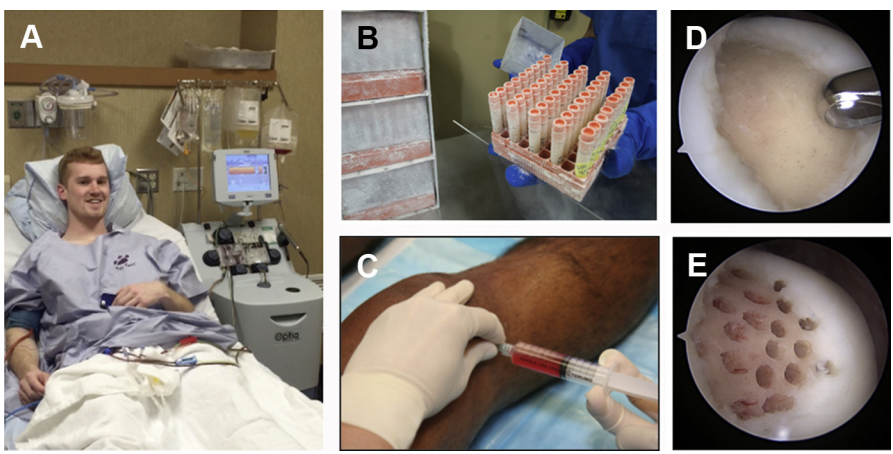

Fig. 6. After aphaeresis harvest (*A*), aliquoting and cryopreservation of mobilized PBSCs (*B*) allow for harvest at one time point and multiple injections (*C*) after arthroscopic subchondral drilling of large cartilage defects (*D*, *E*). (*From* Saw KY, Anz A, Jee CSY, et al. High tibial osteotomy in combination with chondrogenesis after stem cell therapy: a histologic report of 8 cases. Arthroscopy 2015;31(10):1909–20; with permission.)

clinical outcomes scores did not reveal superiority. On average, each stem cell injection in the intervention group contained 8 million stem cells.[50] This group recently published a case series combining the cartilage procedure with high tibial osteotomy.[47] Repair cartilage in this combination produced the best histology to date, and when graded with the ICRS scoring system, the cartilage repair score approached 95% of a normal articular cartilage control (**Fig. 7**).[47] Removing the deforming force responsible for cartilage wear is a key lesson learned. Similar encouraging results have been seen in 2 additional case series involving PBSC and one comparative study of open implantation of PBSC to BMC.[51–53] A multicenter, randomized study is underway in the United States with an IND Application reviewed and approved by the FDA.

A similar technology involving adipose-derived cells for cartilage repair is emerging from a group out of South Korea. Studies initiated with harvesting adipose from the infrapatellar fat pad and settled with liposuction harvest from the buttock region.[54] The methodology for the group involves processing the tissue with centrifugation and a collagenase to digest tissue. It reliably produces 4 million ADSCs from 120 mL of lipoaspirate. The group has investigated one administration time point via intra-articular injection, arthroscopic implantation without a scaffold with PRP, and arthroscopic implantation with a fibrin scaffold. Arthroscopic implantation with a fibrin scaffold has proven safe and the most effective method for administration of this cell product. They have shown that it can improve the clinical results of simple arthroscopic debridement, marrow stimulation, and osteotomy. Comparative study to additional cartilage repair technologies is lacking. This group has reported significant clinical and morphologic improvement when evaluated with MRI; yet histologic results have shown room for further development. These investigators have determined that older age, higher body mass index, and a larger defect size were negative predictors in all studies.[55–62]

THE FUTURE OF ORTHOBIOLOGICS: REGULATORY EVOLUTION

Historically, the FDA has been the global leader of medical regulation. Industrialized nations including but not limited to the European Union, Canada, and Australia have

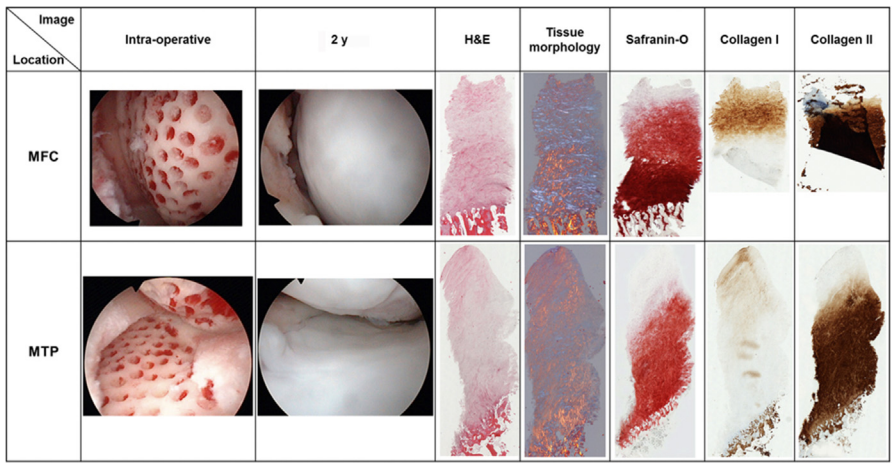

Fig. 7. Findings of second-look arthroscopy and histologic assessment of medial femoral condyle (MFC) and medial tibial plateau (MTP) at 2 years in a 49-year-old male patient. (*From* Saw KY, Anz A, Jee CSY, et al. High tibial osteotomy in combination with chondrogenesis after stem cell therapy: a histologic report of 8 cases. Arthroscopy 2015;31(10):1909–20; with permission.)

traditionally followed their guidance. The strict methods applied by the FDA have created a vacuum as technologies around stem cells have emerged. Although a few clinicians have taken advantage of underdeveloped countries to offer products not available in the United States, including cultured cells and cells from placenta tissue, this is not the norm outside of the United States in developed countries nor in the global stem cell community. The developed world sees the need for regulation in this space to protect vulnerable patients from unproven technologies and regulatory evolution is the key to translating these technologies to patient care.[63]

Regulatory evolution is the future and has begun. In 2014, Japan differentiated stem cell therapies from other pharmaceuticals by referring to these cell treatments as "regenerative medicine products." A new approval system was created and allowed early observed commercialization with reimbursement following a much less demanding safety and efficacy review. With this less demanding system, developing therapies can financially support some of the final most expensive clinical trials through early observed commercialization. With this change in regulation, Japan has positioned them to be leaders in this expanding field of research and development.

In March 2016, an attempt to evolve the US approval system was made. The Reliable and Effective Growth for Regenerative Health Options that Improve Wellness (REGROW) Act was proposed both to the United States Senate and to the House of Representatives and proposed a change in regulation that mirrored Japan's regulatory change. The REGROW Act proposed an addition to the PHSA, section 351B, to specifically address emerging technologies. Section 351B would have allowed for a conditional approval after certain developmental milestones. Specifically, following appropriate animal studies, completion of phase 1 testing, and early results of phase 2 testing, a conditional approval would have been granted to allow the sponsor of the therapy to treat patients and market the therapy during a 5-year trial period. At the end of the 5-year trial, the sponsor would apply for approval of the product as a biologic product. The goal of the addition would be to lower the initial financial hurdle of premarket development steps while still requiring the technologies to prove safety and efficacy.[64]

In late 2016, the discussion and direction of the REGROW Act became enveloped in the 21st Century Cures Act.[64] The 21st Century Cures Act is a bill that was first introduced into the US House of Representatives in January 2015, passed by the House in January 2016, passed by the Senate in October 2016, and signed by President Barack Obama in December 2016. This Act was supported and influenced by large pharmaceutical organizations and opposed by consumer organizations. Through the process, there was discussion about the creation of 351B; however, this was opposed by biopharmaceutical representatives. Instead of creating the 351B pathway, the 21st Century Cures Act created the Regenerative Medicine Advanced Therapy (RMAT) Designation. RMAT designation can be requested by technology sponsors concurrent with an IND application or as an amendment to an IND application. RMAT eligibility is based on 3 conditions: (1) the product is a regenerative medicine therapy, which is defined as a cell therapy, therapeutic tissue engineering product, human cell and tissue product, or any combination product using such therapies or products, except for 316 products; (2) the product is intended to treat, modify, reverse, or cure a serious or life-threatening disease or condition; and (3) preliminary clinical evidence indicates that the drug has the potential to address unmet medical needs for such disease or condition. The FDA upon determination that the technology meets the requirements allows for the treatment to enter one of the FDA's 4 expedited programs for serious conditions.[65] In addition, under certain circumstances, the Act allows for companies to use observational studies, insurance claim data, patient input data, and level V

evidence as opposed to traditional drug trial design.[66] Time will determine whether the RMAT designation is sufficient evolution to improve the translation potential for orthobiologics; however, it is likely that further refinement of the regulation pathway and future legislation or executive direction will be necessary. It is important for orthopedic clinicians and leaders to understand the current regulatory environment and progress in order to participate in the refinement.

SUMMARY

Although the past is marked by murky regulation and the market of unproven treatments, the future of biologics within orthopedics is brighter and clearer with development. Through the developmental pyramid, PRP has proven that it will continue to be a part of the treatment of OA and appears close to an FDA approval for this indication. Similar progress in the biology of ACL surgery is being made, with studies showing the value of an internal splint, scaffolds, and biologic enhancement. Emerging cartilage repair technologies have the largest pyramid of development and are progressing through the FDA approval pathway. In summation, a key has been and will continue to be the developmental process, and reviewing recent paths provides an excellent roadmap for similar emerging therapies.[26] The future will require regulatory involvement and the developmental process both keys for widespread acceptance, payer reimbursement, and accepted clinical application. In light of this fact, regulatory bodies and payers must evolve to expedite the process.

Clinical practice should not outpace evidence regarding safety and efficacy. It is important to remember that patients do represent a vulnerable population, and influencing forces in the orthobiologic space include hope, hype, logistics, and truth. Technologies without transparent development will fade and be replaced by those that performed the necessary steps of development. The orthopedic community must remain grounded in evidence and truth, instead of seeking to profit on the vulnerability of patients by marketing unproven treatments. There is a thick gray area when applying orthobiologics. To navigate, providers should review FDA guidance documents, evaluate the evidence behind technologies, and last, consider the physical risk to the patient and the judicial/regulator risk to the provider. As always, the endless pursuit of well-designed clinical trials and animal studies remains the future for our understanding, and there always remains more to learn. In this space, it is key to stay green and remember: “If you are green, you are still growing. If you are ripe, you are next to rotten.”

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