

Is the Foundation of Regenerative Orthobiologics and Aesthetics Built on Mechanistic Manipulation?

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Submitted: 2026, Jun 01; Accepted: 2026, July 23; Published: 2026, July 29

Citation: Martin, S. M. (2026). Is the Foundation of Regenerative Orthobiologics and Aesthetics Built on Mechanistic Manipulation?. *Adv Bioeng Biomed Sci Res*, 9(1), 01-14

Abstract

Orthobiologic and aesthetic biostimulatory injectables hold a privileged regulatory and commercial position in the orthobiologic and aesthetic injectable markets. Most common amongst these injectable products are Platelet-Rich Plasma (PRP), Bone Marrow Aspirate Concentrate (BMAC), Calcium Hydroxylapatite (CaHA; Radiesse®), and Poly-L-Lactic Acid (PLLA; Sculptra®). Currently, PRP qualifies for Investigational New Drug (IND)-exempt Randomized Controlled Trial (RCT) access as a minimally manipulated, homologous-use autologous biologic. CaHA and PLLA have received FDA clearance for aesthetic applications largely due to mechanistic claims of neocollagenesis and fibroblast biostimulation. BMAC has widespread off-label clinical usage due to clinical literature claims of mechanistic growth-factor and mesenchymal stem cell presence. The purpose of this perspective review is to highlight a foundational mechanistic attribution error in the previous clinical literature of these four orthobiologic and aesthetic injectables. The previous *in vitro* findings that claimed proliferative, chondrogenic, and neocollagenic effects of these injectables incorporated Fetal Bovine Serum (FBS) or fetal calf serum into the cell culture models. FBS does not have a functional analog in human synovial fluid, bone marrow, or dermal interstitium and is not commercially available to clinicians as an injectable product. The reagent largely responsible for the mechanistic signal produced in these previously conducted clinical trials is absent from clinical practice and cannot be replicated in human patients. Burgeoning state regulatory frameworks around which new Stem Cell and Regenerative Therapy Laws are drafted should therefore anticipate and invite both comparative IRB-approved research and expanded clinical use as paired conditions of the same legislative authority.

Keywords: Platelet-Rich Plasma, Bone Marrow Aspirate Concentrate, Calcium Hydroxylapatite, Poly-L-Lactic Acid, Biostimulators, Fetal Bovine Serum, Mechanistic Laundering, Comparative IRB-Approved Trials.

1. Introduction

Over the past two decades, a clinical field has emerged around the premise that autologous and biostimulatory injectables-Platelet-Rich Plasma (PRP), Bone Marrow Aspirate Concentrate (BMAC), Calcium Hydroxylapatite (CaHA), Poly-L-Lactic Acid (PLLA)- recapitulate *in vivo* the proliferative, chondrogenic, anti-inflammatory, and neocollagenic effects observed when those products or their proxies are applied to cells in laboratory cell cultures. This foundational premise is the attribution error motivating the advancements of clinical trials within the fields of these orthobiologic and aesthetic biostimulatory injectables.

This misattribution is largely responsible for the regulatory classifications that grant Investigational New Drug (IND)-exempt Randomized Control Trial (RCT) access to PRP, the FDA clearances that permit CaHA and PLLA for aesthetic indications, and the off-label expansion of BMAC into orthopedic practice. This has ultimately led to the multibillion-dollar cumulative annual market valuation of these orthobiologics and aesthetics injectable market [1].

The misleading results of these clinical trials have not been independently replicated or rigorously tested in simulated

cell models applicable to humans. The foundational *in vitro* experiments that established the mechanistic vocabulary of this field were performed in cell-culture systems supplemented with Fetal Bovine Serum or fetal calf serum, typically at concentrations of five to twenty percent [2]. The clinical products marketed on the strength of those experiments are autologous, in the case of PRP and BMAC, or implanted into FBS-free human dermal tissue in the case of CaHA and PLLA. The translational inference that the *in vivo* product reproduces the *in vitro* signal therefore depends on a step that has not been demonstrated: that the observed *in vitro* effect is attributable to the test agent rather than to FBS itself, to xenogeneic protein-mediated activation of the cells in the system, or to synergy between the test agent and FBS that is not naturally occurring in humans [3].

This significant attribution error is a considerable cause for concern. A cell-culture system in which the test condition contains the test agent plus FBS, and the control condition contains FBS alone, can establish that the test agent adds an increment of activity above the FBS-driven baseline. It cannot establish that the test agent will produce comparable activity in the FBS-free environment of an osteoarthritic joint, an injured tendon, or the human dermis [4]. Doing so would require xenogeneic-free replication, which has yet to be achieved for the four previously mentioned autologous and biostimulatory injectables [5].

In response to these misleading foundational claims, we propose the term “mechanistic laundering” to describe a recurring pattern in this literature in which an *in vitro* signal of mixed provenance is attributed cleanly to a test agent; a clinical product is built around that attribution; and clinical trials are designed using subjective outcome measures — Visual Analog Scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Global Aesthetic Improvement Scale (GAIS) — that cannot falsify the proposed mechanism even when they document a clinical effect. Individual outcomes may be considered accurate, but the causal attribution is incorrect. Thus, the conversion of a mixed-provenance *in vitro* signal into a clean *in vivo* claim, without the intermediate verification step is required.

The foundational claims citing significant improvement in models with FBS consistently have three sequential failures of attribution discipline. First, the results of the *in vitro* experiments attribute an FBS-confounded signal to the test agent [6]. Second, subsequent citing papers reproduce the attribution without re-examining the methodological foundation [7,8]. Third, regulatory submissions, clinical practice guidelines, and marketing claims inherit the unverified attribution as an established mechanism. By the time the claim is challenged, the citation chain is long enough that no single paper bears the weight of the original error, and the field's clinical and commercial momentum makes re-examination commercially and professionally costly.

The regulatory consequences of mechanistic laundering are an asymmetry that this perspective review will examine as some of

the most clinically important features of the current orthobiologic and aesthetic injectable landscape. PRP holds IND-exempt RCT access under FDA homologous-use classification due to these inadequately verified mechanistic claims. CaHA and PLLA, on the strength of similar claims, hold clearance for aesthetic indications. Additionally, lyophilized Wharton's Jelly (WJ), in which functional homology to articular cartilage has been demonstrated through biochemical, biomechanical, and immunologic congruence by a standard that the orthobiologic literature failed to meet, is classified as a Section 351 product and is excluded from RCT access without IND clearance [9].

The asymmetry is not minor and should not be considered as such. It is the difference between a product class whose mechanistic foundation was accepted on inherited claims and a product class whose mechanistic foundation was independently demonstrated being denied the same regulatory access. Resolving the asymmetry requires either re-examination of the foundational literature underpinning the privileged classifications, extension of comparable access to product classes whose mechanism has been demonstrated to the same or higher standard, or both. This perspective review will aim to: (1) Document the FBS confounding in the foundational mechanistic literature for PRP, BMAC, CaHA, and PLLA, (2) Trace the citation chain by which compromised *in vitro* work was elevated into clinical justification and regulatory posture, (3) Survey the clinical reproducibility data and demonstrate the gap between mechanistic claim and clinical outcome across the four categories, (4) Position the methodology applied in our 2025 work on lyophilized WJ as the methodological counter-example, (5) Detail IRCM-2025-468 (KOA-001), the first IRB-approved RCT comparing single-spin red blood cell (RBC)-sparing PRP versus lyophilized WJ in moderate-to-severe knee osteoarthritis, as the operational answer to the comparative question the field has avoided, and (6) Propose minimum translational standards: xenogeneic-free *in vitro* replication, intra-tissue biomarker confirmation of proposed mechanism *in vivo*, and outcome measures capable of falsifying mechanism rather than only symptom [9].

2. Methodological Framework: The FBS Confound and the Standard of Xenogeneic-Free Replication

2.1. Fetal Bovine Serum & Its Role in Cell Culture

Fetal Bovine Serum is the serum fraction of blood collected from bovine fetuses at the time of maternal slaughter. It is the most widely used supplement in mammalian cell culture, included in growth media at concentrations of 5 to 25% (v/v) to support cell attachment, proliferation, and viability [10]. Its biological activity is derived from a poorly characterized and lot-variable mixture of components: albumin, globulins, transferrin, growth factors including Transforming Growth Factor beta (TGF- β), Platelet-Derived Growth Factor (PDGF), Insulin-like Growth Factor 1 (IGF-1), Epidermal Growth Factor (EGF), and Fibroblast Growth Factor (FGF); hormones; lipids; trace elements; and a substantial fraction of unidentified proteins, peptides, and extracellular vesicles [11].

Despite the variety of components, FBS is not a passive nutritional supplement. It is a complex bioactive cocktail whose composition includes the very growth factors and signaling molecules that orthobiologic products claim to deliver. PDGF, TGF- β , IGF-1, and EGF — the growth factors marketed as the active mediators of PRP's therapeutic effect — are present in significant concentrations of FBS itself [12]. The previously alluded chondrogenic and anabolic signaling capabilities of BMAC occur within the same TGF- β and IGF-1 pathways that FBS activates by virtue of containing these growth factors [13]. Finally, the fibroblast proliferation claimed for CaHA and PLLA biostimulation also operates through PDGF and FGF signaling, again present in FBS [12,14].

2.2. Identification & Significance of the Attribution Error

We distinguished two specific failures of attribution discipline in the literature surveyed by this perspective review. Upstream attribution failure occurs when an *in vitro* mechanistic claim is established in an FBS-supplemented system without xenogeneic-free replication, and the claim is subsequently cited in regulatory, clinical, or marketing contexts as an established mechanism. PRP, BMAC, CaHA, and PLLA all exhibit this failure to varying degrees, and the body of this review documents each case. Downstream attribution failure occurs when a clinical signal — typically a positive outcome on a subjective measure such as VAS or WOMAC — is attributed to the proposed mechanism without intra-tissue biomarker confirmation that the mechanism is operating *in vivo*. A reduction in WOMAC score following PRP injection does not, by itself, establish growth-factor-mediated cartilage anabolism as the causal mechanism. Similar improvements may arise from placebo effects, non-specific effects of intra-articular injection, potential mechanical effects of injected joint volume, anti-inflammatory responses unrelated to cartilage regeneration, and regression to the mean; therefore, mechanistic attribution requires evidence beyond clinical outcome improvement alone [15]. Without synovial cytokine, biomarker, or imaging data demonstrating that the proposed mechanism is operating, the clinical signal cannot adjudicate among these possibilities.

The methodological consequence of FBS supplementation in mechanistic *in vitro* studies of orthobiologic and biostimulator products is drastic. Consider the experimental design typical of the foundational literature in each category: a target cell type — chondrocyte, mesenchymal stem cell, fibroblast — is plated in growth medium containing 5-10% FBS. The test agent — PRP release, BMAC supernatant, CaHA microsphere conditioned medium, PLLA particle conditioned medium — is then added. A control condition contains the medium with FBS but without the test agent. Outcome measures — proliferation rate, collagen synthesis, glycosaminoglycan deposition, cytokine expression — are then compared between conditions. This design can establish that the test agent adds an increment of activity above an FBS-driven baseline. It cannot establish three claims that the citation chain routinely makes: (1) That the test agent is the active mediator of the observed effect, rather than the test agent operating synergistically with FBS components, (2) that the magnitude of

the test agent's effect would be preserved in the absence of FBS, and (3) that the *in vivo* environment — osteoarthritic synovial fluid, dermal interstitium — provides the same permissive milieu as FBS-containing culture medium, and therefore that the *in vitro* effect predicts the *in vivo* effect [12,16]. Each of these three claims requires a different and more rigorous experimental design than the FBS-supplemented baseline standard.

While the foundational mechanistic experiments for PRP, BMAC, CaHA, and PLLA depend on FBS as a culture supplement, it is considered a research-grade reagent and cannot be purchased by clinical providers through reputable purchasing organizations (i.e., Cardinal Health or McKesson). It is not in the supply chain of any practicing physician injecting PRP into an osteoarthritic knee, aspirating bone marrow for BMAC, or implanting CaHA or PLLA into the dermis. The substance that drove the original mechanistic signal is structurally absent from clinical practice. The *in vivo* environment into which these products are injected does not contain FBS or any functional analog of it [17]. The concentrated fetal-derived growth factor and bioactive milieu that supported the *in vitro* signal has no counterpart in the adult human tissues these products enter. The bench condition cannot exist at the bedside in principle, not merely in practice. A clinician who wished to confirm that PRP release stimulates chondrocyte matrix synthesis under the conditions the foundational literature reports cannot do so at the point of care, because the foundational conditions cannot be reproduced at the point of care. The claim is not merely unverified. The claim is unverifiable by the persons clinically responsible for the patients to whom the products are administered.

Thus, we propose the term “irreproducible by design” to identify this lapse in reproducible results within patients. The term does not impute intent to the original investigators, who were operating within the cell-culture conventions of their era. It names the inherited consequence: a field whose foundational mechanistic warrants cannot be tested by the clinicians who rely on them, in the patients on whom they act. The mechanistic laundering is the propagation mechanism. Irreproducibility by design is the result. The two together define the methodological posture this review challenges.

2.3. The Xenogeneic-Free Replication Standard

The standard of evidence that would resolve the FBS confound is xenogeneic-free replication: the demonstration that a mechanistic effect attributed to a test agent in FBS-supplemented culture is reproduced when the same agent is tested under conditions free of bovine or other xenogeneic serum. This standard has been adopted, with varying rigor, in stem cell manufacturing for clinical translation, where regulatory authorities have increasingly required serum-free or human-platelet-lysate-supplemented culture for cells intended for human administration [18].

However, the orthobiologic and aesthetic biostimulator literature has not been held to this same standard. The foundational *in vitro* work establishing the mechanisms of PRP, BMAC, CaHA,

and PLLA predates the widespread regulatory adoption of xenogeneic-free standards, and the citation chain that propagates the original mechanistic claims has not, in the intervening decades, undertaken systematic re-examination under the newer standard [12]. Consequently, products marketed and reimbursed on the strength of mechanistic claims would, if those products were stem cell therapies subject to current regulatory expectations, be required to demonstrate the same mechanisms under conditions the foundational literature did not employ.

2.4. Correcting the Attribution Error

The standard of attribution disciplines this review proposes is not novel. It is the standard applied in our 2025 work on lyophilized WJ, and it is the standard applied throughout regulatory cell-therapy manufacturing [9]. It requires three demonstrations before a mechanistic claim is applied to clinical justification. First, in vitro replication is free of xenogeneic confound. The mechanism is reproduced in autologous serum, human-platelet-lysate, or chemically defined serum-free conditions. Second, in vivo biomarker confirmation must occur. The proposed mechanism is demonstrated to operate in the target tissue, by direct measurement of the relevant cytokine, growth factor, matrix component, or imaging biomarker after administration. Finally, the outcome measures must be capable of falsifying the mechanism. Clinical endpoints must be able to distinguish the proposed mechanism from competing explanations — placebo, mechanical effect, non-specific inflammation, repeated-injection effect. The remainder of this review documents the failure of the orthobiologic and aesthetic biostimulator literature to meet these three standards for PRP, BMAC, CaHA, and PLLA, and contrasts that failure with the demonstration of these standards for lyophilized WJ in our prior work and in the design of IRCM-2025-468 (KOA-001) [9].

3. Platelet-Rich Plasma

3.1. PRP & Its Mechanistic Claim

Platelet-Rich Plasma is the supernatant fraction of autologous whole blood centrifuged to concentrate platelets at multiples of baseline circulating concentration, with the resulting platelet-enriched plasma injected into joints, tendons, ligaments, or dermis. The mechanistic claim, established in the foundational literature of the late 1990s and early 2000s and propagated essentially unchanged through the subsequent two decades of clinical literature, is that platelet degranulation at the injection site releases a cascade of growth factors — Platelet-Derived Growth Factor, Transforming Growth Factor beta, Vascular Endothelial Growth Factor, Insulin-like Growth Factor 1, Epidermal Growth Factor, basic Fibroblast Growth Factor — which stimulate cellular proliferation, matrix synthesis, angiogenesis, and tissue repair in the target [19,20].

3.2. The Foundational In Vivo Literature & FBS Confound

The chondrocyte and tenocyte proliferation and matrix-synthesis literature that established PRP's claimed regenerative effect on cartilage and tendon was performed almost exclusively in cell-culture systems supplemented with FBS at 5-20% [21,22]. The experimental design across this literature is broadly uniform:

primary chondrocytes or tenocytes isolated from human or animal donors are plated in growth medium containing FBS; the test condition adds PRP release, platelet lysate, or PRP-conditioned medium at varying concentrations; and the control condition contains the same FBS-supplemented medium without the test agent [23]. The outcome measures of proliferation, glycosaminoglycan deposition, type II collagen expression, and aggrecan synthesis are compared between conditions.

The methodological consequence is that every effect reported in this literature represents the increment above an FBS-driven baseline that itself contains TGF- β , PDGF, IGF-1, and FGF — the same growth factors PRP is claimed to deliver [24]. The literature has not, with the rigor the clinical claims would require, replicated these effects under xenogeneic-free conditions: in autologous human serum, in human platelet lysate, or in chemically defined serum-free media. Where such replication has been attempted, the magnitude of the PRP effect has been reported as attenuated or absent, and the field has not assimilated those findings into the consensus mechanistic narrative [25].

3.3. The Pro-Inflammatory Objection

A second methodological problem in the PRP mechanistic literature is the framing of PRP as anti-inflammatory or regenerative on the basis of in vitro proliferation data, when PRP is mechanistically a pro-inflammatory preparation in its acute phase. Platelet degranulation at the injection site releases not only the anabolic growth factors marketed in the clinical literature, but also pro-inflammatory mediators: thromboxane A₂, serotonin, histamine, complement activators including C3a and C5a, in addition to the precursors and amplifiers of Interleukin-1 beta signaling [26,27]. The early-phase response to PRP injection is the wound-healing inflammatory cascade, with neutrophil and macrophage recruitment, local vasodilation, and amplification of the very cytokines — IL-1 β , IL-6, TNF- α — that the orthobiologic literature otherwise characterizes as pathological in osteoarthritic joints.

The field's marketing of PRP as anti-inflammatory depends on the premise that the later-phase anabolic signaling dominates the earlier-phase inflammatory signaling at the tissue level. This premise has not been demonstrated by intra-articular cytokine measurement in human trials. Synovial fluid cytokine profiles after PRP injection in human osteoarthritic knees have been reported only sparsely, and the available data do not support a consistent shift toward anti-inflammatory cytokine dominance [28].

3.4. The Clinical Reproducibility Gap

The clinical literature on PRP for knee osteoarthritis is large, heterogeneous, and methodologically uneven. Individual randomized controlled trials report effect sizes ranging from clinically meaningful to null [15,29]. Meta-analyses also report modest pooled effects with high heterogeneity [30]. The 2019 Osteoarthritis Research Society International (OARSI) guidelines for the non-surgical management of knee osteoarthritis explicitly

do not recommend PRP for routine clinical use, citing insufficient evidence of efficacy [31].

The gap between the mechanistic claim — growth-factor-mediated cartilage anabolism producing structural and symptomatic improvement — and the clinical outcome data is the predictable consequence of the upstream attribution failure. A mechanism established only in FBS-confounded *in vitro* systems, never independently confirmed under xenogeneic-free conditions, never demonstrated *in vivo* by intra-articular biomarker measurement, will not reliably predict clinical effect. The PRP clinical literature is the result of that prediction failure made over 20 years of clinical practice.

As mentioned previously, PRP holds IND-exempt RCT access under FDA classification as a minimally manipulated, homologous-use autologous biologic. This classification was extended in part on the strength of the mechanistic claims documented in section 3.1, and it has enabled an extensive clinical research enterprise that has produced the reproducibility gap documented in section 3.4. The conditions under which PRP's signature *in vitro* effects were demonstrated cannot be reproduced in the clinic, and the conditions inside the joint or tendon do not approximate those *in vitro* conditions. The mechanistic claim is irreproducible by design at the point of care.

4. Bone Marrow Aspirate Concentrate

4.1. BMAC & the Mechanistic Claim

Bone Marrow Aspirate Concentrate is the centrifuged fraction of autologous bone marrow aspirate, typically harvested from the posterior iliac crest, injected into joints, intervertebral discs, or tendon/ligament insertion sites. The mechanistic claim is twofold: that BMAC delivers mesenchymal stem cells (MSCs) capable of differentiating into chondrocytes, osteoblasts, or tenocytes at the target site; and that BMAC delivers a growth factor and cytokine milieu — including the same PDGF, TGF- β , VEGF, and IGF-1 invoked for PRP — that supports tissue repair through paracrine signaling [32].

4.2. The Foundational MSC Literature in BMAC & the FBS Confound

The mesenchymal stem cell differentiation paradigm that underwrites the BMAC mechanistic claim was established in cell-culture systems uniformly supplemented with FBS [33]. The foundational chondrogenic, osteogenic, and adipogenic differentiation protocols — the demonstrations that plastic-adherent bone marrow mononuclear cells can be induced to express chondrocyte, osteoblast, or adipocyte markers under appropriate stimulation — were performed in FBS-containing media [33]. The FBS concentrations ranged from 10-20% during the expansion phase, with additional supplementation of specific differentiation cocktails during the induction phase [33].

The methodological consequence in BMAC's case is more severe than in PRP's, for two reasons. First, the MSCs studied in the

foundational differentiation literature were not BMAC. They were plastic-adherent, culture-expanded, passaged cells. The clinical product injected as BMAC is a single-spin or double-spin centrifuged aspirate containing a heterogeneous nucleated cell population of well under 1% of true MSCs and under 0.1% of the total nucleated [34]. “True MSCs” are defined by the ISCT criteria of plastic adherence, surface marker expression, and trilineage differentiation potential [35,36].

Second, the cells that would, by these counts, be present in BMAC have not been demonstrated to differentiate into chondrocytes *in vivo* at the injection site [35]. The differentiation paradigm was established *in vitro* under conditions of plastic adherence, prolonged culture expansion, specific media supplementation, and FBS support. The injection of a heterogeneous, unexpanded, FBS-naïve aspirate into an inflamed osteoarthritic joint is not the experimental condition under which differentiation was demonstrated. The mechanistic claim therefore inherits two layers of attribution failure: an FBS-confounded foundational paradigm and an unjustified extrapolation from culture-expanded MSCs to clinical BMAC.

4.3. The Clinical Reproducibility Gap

The BMAC clinical literature for knee osteoarthritis is smaller than the PRP literature but exhibits a similar pattern: heterogeneous effect sizes, methodologically uneven trials, and an absence of evidence demonstrating the proposed mechanism (MSC-mediated cartilage regeneration) *in vivo* [36-38]. Imaging and histologic evidence of cartilage regeneration after BMAC injection in human knees is sparse and methodologically limited. Symptomatic outcome measures (i.e. VAS, WOMAC) show variable improvement that is consistent with the proposed mechanism, with placebo, with anti-inflammatory effects of the injection procedure, and with the same set of competing explanations that complicate interpretation of the PRP literature [15].

4.4. The Regulatory Posture

BMAC occupies a regulatorily ambiguous position: it is widely used in off-label orthopedic practice under the same minimally manipulated, homologous-use framework that classifies PRP, yet its classification has been subject to enforcement discretion and periodic FDA scrutiny rather than consistent regulatory clarity. The mechanistic claim that justifies its homologous-use classification — that bone marrow's natural role in supporting tissue repair is preserved when bone marrow is injected into damaged joint tissue — depends on the MSC differentiation paradigm whose foundational attribution failures are documented in section 4.2.

4.5. Irreproducibility by Design

The issue of irreproducibility for BMAC is the most severe of the four categories examined in this review, because it operates at two compounding levels. At the first level, the MSC differentiation paradigm itself was established in FBS-supplemented, plastic-adherent, culture-expanded systems that no practicing physician can recreate at the bedside. At the second level, the clinical product

is not the MSC population from which the paradigm was derived; it is an unexpanded heterogeneous aspirate in which the cells of interest constitute a fraction of 1% of the total nucleated population [34]. The clinician injecting BMAC into a patient's joint cannot verify that the cells responsible for the foundational differentiation signal are present in the injectate in functional numbers, cannot recreate the FBS-supplemented expansion conditions under which the paradigm was demonstrated, and cannot test in vivo whether the differentiation the paradigm asserts is actually occurring at the target site. The mechanistic claim is irreproducible by design at both levels: the reagent and culture conditions of the foundational paradigm, and the cell population that paradigm requires.

5. Calcium Hydroxylapatite and Poly-L-Lactic Acid: The Aesthetic Biostimulator Pair

5.1. Commercial Products & the Mechanistic Claims

Calcium Hydroxylapatite (CaHA; marketed as Radiesse®) and Poly-L-Lactic Acid (PLLA; marketed as Sculptra®) are injectable resorbable particulate suspensions cleared by the FDA for aesthetic correction of facial volume loss. Both are marketed not as volumizing fillers in the classical hyaluronic acid (HA) sense, but as biostimulators. Biostimulator products claim the mechanism of induction of fibroblast activity and endogenous collagen synthesis (neocollagenesis) at the implantation site, producing cosmetic correction that outlasts the resorption of the implanted particles themselves [39,40]

5.2. The Foundational Fibroblast Literature & the FBS Confound

The fibroblast biostimulation claim underwriting both CaHA and PLLA was established in cell-culture systems supplemented with FBS at 5-10% [41]. The experimental design across this literature is uniform: human dermal fibroblasts are plated in FBS-containing growth medium; the test condition exposes the fibroblasts to CaHA microspheres or PLLA particles or to conditioned medium from particle-exposed cultures; and the control condition contains the same FBS-supplemented medium without particles [41,42]. The outcome measures, which consist of fibroblast proliferation, type I and type III collagen gene expression, and extracellular matrix deposition, are compared between conditions.

The methodological consequence is the same FBS confound documented in both PRP and BMAC. The test agent's effect is measured as an increment above an FBS-driven fibroblast baseline that itself contains PDGF, TGF- β , and FGF — the growth factors that drive fibroblast proliferation and collagen synthesis [43]. The literature has not, to a standard adequate to support the clinical and regulatory claims, replicated these effects under xenogeneic-free conditions.

5.3. The Foreign Body Response Absent from Clinical Literature

A second and arguably more important methodological problem in the CaHA and PLLA mechanistic literature is the framing of the in vivo tissue response as biostimulation when the response is

mechanistically a foreign body reaction. Implantation of insoluble particulate material into dermal tissue elicits a stereotyped foreign body response: macrophage recruitment, giant cell formation around the particles, and fibrotic encapsulation [44]. The foreign-body response produces fibrotic extracellular matrix characterized by altered collagen organization resembling scar tissue rather than regeneration of native dermal architecture. Early matrix deposition is enriched in type III collagen, whereas native dermis is predominantly composed of highly organized type I collagen [44,45].

The clinical cosmetic effect is demonstrable. The mechanism is primarily volumetric, driven by the implanted particles and the resulting foreign-body response, including fibrotic encapsulation, together with subsequent late-phase collagen remodeling. Calling this mechanism neocollagenesis or biostimulation describes the histologic outcome accurately at the most superficial level and obscures the mechanism at every level below. The marketing reframe from controlled chronic foreign body response to biostimulatory collagen induction is, by the analytical frame of this review, mechanistic laundering in its clearest form.

5.4. The Regulatory Consequence

CaHA and PLLA hold FDA clearance for aesthetic indications. Their regulatory dossiers include the fibroblast biostimulation mechanistic claims previously mentioned. The clinical efficacy of both products for cosmetic volume correction is established [46,47]. What is not established, and what the regulatory posture and marketing nonetheless assert, is that the mechanism is biostimulatory neocollagenesis rather than controlled chronic foreign body response with associated fibrosis. The clinical effect does not require the proposed mechanism to be true. However, the regulatory and marketing claims do.

5.5. The Surgical Readout: When the Operating Field is the In Vivo Experiment

The central argument of this section is that clinical reality is the final arbiter of a mechanistic claim, and that the claims surrounding CaHA and PLLA were never tested in the venue that matters. There is a venue in which these claims are now being tested, against the surgeon's will and at the patient's expense: the operative field of the facelift. A deep-plane facelift is, in effect, a direct in vivo inspection of the tissue these products were claimed to regenerate. The dissection depends on the existence of clean, gliding fascial planes and on the orderly release of the retaining ligaments of the face. If CaHA and PLLA produced the organized, native-architecture neocollagenesis that the marketing asserts, the surgeon entering tissue with a history of repeated biostimulator exposure would encounter dermis and subcutis that behave as healthy, well-organized tissue. Yet, that is not what an increasing number of surgeons report encountering.

In a survey of the membership of The Aesthetic Society, approximately half of the responding surgeons reported that a history of repetitive panfacial filler injection increased the difficulty

of performing a subsequent facelift. Amongst the surgeons reporting increased difficulty, the most frequently cited reason was distortion of the tissue planes by scarring, followed by the technical difficulty of raising healthy flaps, with a substantial fraction also reporting increased operative time (Sweis et al., 2023) [47]. The finding most relevant to the present argument is the product-specific one: when respondents were asked which materials caused the most difficulty with subsequent facelift surgery, the semi-permanent and permanent agents — calcium hydroxylapatite and polymethylmethacrylate, with poly-L-lactic acid implicated at a comparable rate — were named far more often than HA, despite HA being used by virtually every surgeon surveyed [48]. Notably, the material that the surgeons did not blame is the one that can be enzymatically removed before they operate.

This is the divergence that the reversibility of the products predicts. Patients with significant prior exposure to Radiesse® and Sculptra® may encounter pre-surgical impediments not experienced by patients treated with highly cross-linked HA [48]. The potential for dissolution differs not in degree but in kind: cross-linked HA, however resistant, remains enzymatically addressable, whereas the fibrotic tissue response induced by calcium hydroxylapatite and poly-L-lactic acid has no dissolution pathway and presents to the surgeon as an established scar [49,50]. The HA can be titrated away with hyaluronidase in the weeks before surgery; the biostimulator-induced fibrosis cannot be titrated away at all. The surgeon inherits it, dissects through it, and is left to achieve an aesthetic result in tissue whose native planes have been effaced.

The surgical experience addresses the mechanistic question raised in section 5.3, which remains unresolved in the histologic literature. The manufacturer-sponsored histology reports that CaHA stimulates an early predominance of type III collagen that matures toward type I over the following months, framed as evidence of orderly dermal remodeling [39]. A 2025 commentary in the *Aesthetic Surgery Journal* challenges precisely the inference drawn from that data, arguing that CaHA functions as a biocompatible scaffold inducing a controlled fibrous response [51]. It is argued that this is tissue repair rather than true regeneration, and that the type III-to-type I transition the marketing cites as maturation is the signature of a scar maturing into dense, functionally inferior connective tissue rather than the restoration of native dermal architecture [51]. The debate over what the biopsy shows is ultimately adjudicated by what the scalpel reveals. The surgeon dissecting a biostimulator-exposed face is reading the only histology that is not confounded by the conditions of its own production: the tissue itself, in the living patient, years after injection. The tissue that reads as scar — adherent, vascularly unreliable, resistant to clean planar release and is the *in vivo* result the foundational *in vitro* literature was never required to produce.

The evidentiary status of this surgical claim must be stated with the same discipline this review demands of the orthobiologic literature it critiques. The survey data is level 5 evidence: practitioner perception, a low response rate, and no objective intraoperative

measurement isolating CaHA or PLLA exposure from confounding HA, fat, and repeat-injection history [48]. However, the histologic debate is unresolved. There is yet to be a prospective controlled study to quantify operative time, flap viability, or aesthetic outcome as a function of biostimulator exposure with the confounders removed. By the three-part standard previously proposed in section 2.4, the surgical claim is presently at the first stage only — a clinically observed signal awaiting the intra-tissue and outcome-level confirmation that would convert observation into demonstration. To assert more than that would be to commit, in the opposite direction, the very attribution error this review documents. The claim made here is therefore the disciplined one: that the operating field is the correct venue in which to test these mechanisms, that the early signal from that venue is consistent with the foreign-body-repair mechanism of section 5.3 and inconsistent with the clean neocollagenesis of the marketing claim, and that the prospective surgical study isolating biostimulator exposure is the experiment the field now owes its patients. It follows the same experimental approach, conducted in the same spirit as IRCM-2025-468.

Clinicians are struggling to achieve real-world outcomes for their patients because the foundational case for these products was built from *in vitro* experiments and never reproduced *in vivo* by the persons clinically responsible for the result. The aesthetic surgeon laboring through effaced tissue planes to recover a natural facial contour, and in some practices declining to operate at all, is suffering from the cost of that omission. A product marketed and sold as restoring the architecture of youthful tissue is, in these patients, obstructing the definitive surgical means of restoring it. That is not a marketing claim failing quietly. It is a laundered mechanism failing in the operating room, where the patient is on the table, and the bench condition that generated the original claim is nowhere to be found.

5.6. Irreproducibility by Design

The fibroblast biostimulation claim for CaHA and PLLA carries a particular irreproducibility burden, because the clinical setting in which these products are administered — the aesthetic injection suite — is the setting furthest from the cell-culture conditions under which the claim was originally established. The injector inserting CaHA or PLLA into a patient's dermis has no instrument by which to confirm that the dermal fibroblasts of an aging adult patient respond as the cultured neonatal dermal fibroblasts in the foundational studies did, no means to replicate the FBS-supplemented growth medium in which those cells were primed, and no way to distinguish the neocollagenesis the marketing asserts from the fibrotic encapsulation the histology shows. The mechanistic claim is irreproducible by design, and the clinician's only available verification is the patient's own subjective cosmetic outcome — a measure that does not require the proposed biologic mechanism to be true.

6. The Methodological Counterexample: Lyophilized Wharton's Jelly

6.1. Lyophilized Wharton's jelly serves as a Model for Mechanistic Claims

The four product categories previously examined share a common methodological flaw: mechanistic claims established in FBS-confounded in vitro systems, propagated through citation chains into clinical and regulatory contexts, and not independently verified by the previously proposed three-part standard. A review documenting this pattern is incomplete without an answer to the implicit question it raises: is the standard achievable? Is there a regenerative biologic whose mechanism has been demonstrated by the criteria the orthobiologic literature failed to meet?

The answer, demonstrated in our 2025 work, is lyophilized WJ [9]. The methodology by which functional homology to articular cartilage was established for WJ in that paper is the methodological counterexample this section presents. This is not because WJ is uniquely virtuous among regenerative biologics, but because the standard of evidence applied to it is the standard the rest of the field has not been required to meet. The 2025 paper established the case for IND-exempt RCT access to lyophilized WJ on the basis of functional homology to articular cartilage [9]. The argument did not rest on inherited mechanistic claims from FBS-confounded cell-culture experiments; it rested on three independent and verifiable demonstrations.

6.2. Functional Homology Established by Demonstration, Not Assumption

The 2025 paper examined three essential categories that the foundational literature in the four orthobiologic and aesthetic biostimulatory injectables has yet to establish. First, the concept of biochemical congruence was analyzed. The extracellular matrix composition of Wharton's Jelly was characterized in primary tissue and compared to the matrix composition of articular cartilage. Both tissues contain hyaluronic acid, type I and type III collagen, sulfated proteoglycans, and bioactive mediators including Interleukin-1 Receptor Antagonist (IL-1Ra), Transforming Growth Factor beta, and Tissue Inhibitors of Metalloproteinases (TIMPs) [9]. The congruence is documented by direct tissue analysis, not by cell-culture extrapolation. The second category examined by this paper was biomechanical congruence. The viscoelastic, non-linear stress-strain, and load-redistribution properties of WJ were characterized in primary tissue and compared to those of articular cartilage. The congruence is documented by direct biomechanical testing of the tissue in its native and lyophilized forms, not by inference from cellular behavior in culture. The third and final category explored was the immunologic profile. The immunologic safety of decellularized and lyophilized WJ was characterized in human clinical exposure data, including the absence of meaningful graft-versus-host response or synovial inflammation in intra-articular use [9]. The profile is documented by direct clinical observation, not by inference from in vitro cytokine assays. These three categories are independent in that none of the claims depended on foreign cell culture supplementation (FBS) to argue functional homology or required the inference from cellular behavior in vitro to tissue

behavior in vivo. The mechanistic case for WJ as a structural and biochemical analog of articular cartilage is established by direct measurement of the tissue itself.

6.3. The Regulatory Contradiction

The 2025 paper documented a regulatory asymmetry that the present review now restates with the previously mentioned methodological framework added to it. PRP, on the strength of mechanistic claims established under the FBS-confounded conditions, holds IND-exempt RCT access under FDA homologous-use classification. Lyophilized WJ, whose mechanistic case is established by the direct tissue characterization, is classified as a Section 351 product and is excluded from RCT access without IND clearance.

The asymmetry is not explicable on the basis of evidence quality. The asymmetry is explicable only as the artifact of two different historical pathways through the regulatory system. PRP entered the homologous-use category in an era when mechanistic warrants were accepted on the strength of in vitro literature whose methodological limits had not yet been examined. WJ has been required to meet a higher standard precisely because the regulatory framework has, in the interim, become more cautious about cellular and tissue products without correspondingly reexamining the mechanistic warrants of products previously admitted under the lower standard.

Consequently, regulatory inconsistency is at the expense of clinical research. The product class whose mechanism is more rigorously established cannot be studied in RCTs against the product class whose mechanism is less rigorously established, because only the latter received IND exemption. The overarching clinical question of which product results in better outcomes in patients with osteoarthritis cannot be answered through the standard mechanism of comparative clinical research.

7. IRCM-2025-468 (KOA-001): The Proposed Operational Answer

7.1. Trial Overview

KOA-001, a comparative osteoarthritis knee trial, is a pragmatic, outcome-based, multi-site randomized controlled trial comparing single-spin RBC-sparing PRP versus lyophilized WJ in patients with moderate to severe osteoarthritis (IRB approval #IRCM-2025-468, HHS/OHRP IRB registration IRB00009500). This protocol was designed to address the methodological failures this review documents in the orthobiologic literature. This comparative trial is unique compared to the aforementioned clinical literature due to five design features.

7.1.1. Head-to-Head Comparative Design

The trial directly compares the two products — single-spin RBC-sparing PRP versus lyophilized WJ. This is significant because the regulatory asymmetry has previously prevented these products from being studied against each other. The trial employs a partially blinded design and participant randomization to minimize inflated patient-reported outcomes between the two injectables.

7.1.2. Pragmatic Outcomes

The trial measures patient-reported outcome measures appropriate to the clinical question — pain, function, durability of effect — rather than measures designed to confirm a specific mechanistic hypothesis. Patient-reported outcome measures, including WOMAC, KOOS, and VAS, allowed participants to directly report their perceptions of pain, function, and overall treatment response, ensuring that the assessment reflected the patient perspective rather than solely clinician-reported outcomes. The pragmatic design is in alignment with the goal to determine which product produces better clinical outcomes, not to confirm which mechanistic story is correct.

7.1.3. Pre-Specified Analysis

The statistical analysis plan is pre-specified before unblinding. Thus, preventing the post hoc subgroup analyses and outcome-switching that have characterized portions of the orthobiologic literature. Statistical analysis will be conducted on coded, de-identified datasets where treatment assignment is masked until primary analyses are completed.

7.1.4. International Multi-Site Structure Under IRB Oversight

The trial is conducted under a single IRB of record (IRCM, IRB00009500) registered with the U.S. Office for Human Research Protections, enabling Common Rule-compliant oversight of international and United States sites under a single protocol. The structure permits the comparative trial to proceed in jurisdictions where it can ethically and competently be conducted, while preserving the standard of human subjects' protection required for the resulting evidence to be usable in international peer-reviewed literature and in future regulatory submissions.

7.1.5. Independent of Product-Manufacturer Sponsorship

A distinguishing feature of this trial is the absence of product-manufacturer sponsorship or financial support from entities with a commercial interest in either intervention. By evaluating PRP and WJ under an investigator-initiated framework, the study is designed to minimize potential sponsorship-related influences on study conduct, data interpretation, and reporting. This independent comparative design allows outcomes to be assessed on the basis of patient-reported and clinical measures rather than commercial considerations.

7.2. Scope & Limitations of the Trial

KOA-001 will produce head-to-head comparative clinical outcome data for the two products in moderate-to-severe knee osteoarthritis. It will answer the question of which product produces better clinical outcomes in the populations enrolled. It will not, by itself, validate or invalidate the mechanistic claims of either product. The mechanistic question — whether the proposed in vivo mechanism is actually operating in the target tissue — requires the intra-tissue biomarker measurement specified as the second component of the three-part standard previously proposed, and KOA-001 is not designed primarily as a mechanistic study.

This self-imposed limitation is itself part of the methodological case the present review makes. A clinical trial that distinguishes comparative outcome from mechanistic confirmation and is transparent about which question its design can answer, is a clinical trial conducted by a standard the foundational orthobiologic literature did not meet. The subsequent mechanistic studies that should accompany or follow KOA-001 (synovial fluid cytokine measurement, imaging biomarkers, structural endpoints) are the next stage of the work and are the appropriate place to test the mechanistic claims this review has documented as inadequately verified.

8. Proposed Translational Standards

8.1. The Three Demonstrations

The standard of attribution discipline proposed by this review, applicable to any regenerative biologic or biostimulatory injectable seeking clinical claim or regulatory privilege, comprises three demonstrations.

8.1.1. Xenogenic-Free In Vivo Replication

Any mechanistic claim originating in FBS-supplemented or otherwise xenogenic-contaminated cell-culture experiments must be replicated under conditions free of bovine or other xenogenic serum — in autologous human serum, in human platelet lysate, or in chemically defined serum-free media — before the claim is elevated into clinical justification.

8.1.2. Intra-Tissue Biomarker Confirmation In Vivo

Any mechanistic claim asserting a specific in vivo action (i.e. cytokine modulation, growth factor delivery, matrix synthesis, cellular differentiation) must be confirmed by direct measurement of the relevant biomarker in the target tissue after administration. The clinical signal alone cannot establish the mechanism, because clinical signals are consistent with multiple competing mechanisms.

8.1.3. Outcome Measures Capable of Falsifying Mechanism

Clinical trials supporting a mechanistic claim must include outcome measures capable of distinguishing the proposed mechanism from competing explanations: placebo, mechanical effect of injection, non-specific inflammation, repeated-injection effect, and/or regression to the mean. Subjective patient-reported outcomes alone do not satisfy this standard.

8.2. The Regulatory Implications

If these standards were applied retrospectively to the foundational mechanistic literature of PRP, BMAC, CaHA, and PLLA, the regulatory privileges currently extended to these products on the strength of their mechanistic claims would require reexamination. We do not propose retrospective revocation of those privileges, because the clinical use of these products has been extensive and the disruption of withdrawing access would not be proportionate to the methodological correction required.

We propose instead that the standards apply prospectively to two distinct populations of products: new and existing products. New

regenerative biologics and biostimulatory injectables seeking initial clearance or homologous-use classification should be required to demonstrate mechanism by the three-part standard above. Existing products whose mechanistic claims are challenged by new evidence, including the evidence of inadequate FBS-free replication documented in this review, should be required to demonstrate continued mechanistic validity to maintain the regulatory privileges those claims supported.

A symmetric implication follows for products previously denied regulatory privilege on the grounds that their mechanism was inadequately established. Lyophilized Wharton's Jelly meets the three-part standard by the demonstrations documented in section 6.2 and in our prior work [9]. Its current Section 351 classification and the IND requirement that excludes it from comparative RCT access are not defensible against the standard the present review proposes. The regulatory framework should extend the IND exemption for RCT access to products that meet the standard, regardless of historical category.

9. Discussion

The argument proposed in this review is methodological and regulatory, not clinical. We do not claim that PRP, BMAC, CaHA, or PLLA are clinically ineffective. We claim that the mechanistic warrants extended to these products in the foundational literature were not verified by the standard that the field's own products would require. Additionally, we argue that the regulatory and commercial framework built on those unverified warrants has produced an asymmetry that excludes from RCT access a product class — perinatal-tissue extracellular matrix, specifically lyophilized Wharton's Jelly — whose mechanism has been more rigorously demonstrated.

The clinical outcome literature for the four product categories examined here is the predictable consequence of mechanistic warrants that were never adequately tested. Heterogeneous effect sizes, methodologically uneven trials, and the persistent gap between mechanistic claims and clinical outcomes are not anomalies in this field. They are what one would expect when clinical practice expands on the strength of *in vitro* signals that were never replicated under the conditions of clinical use.

The deeper finding of this review, beyond any individual product category, is structural. The foundational mechanistic literature for PRP, BMAC, CaHA, and PLLA rests on cell-culture experiments performed with FBS. The reagent that produced the foundational signal is, by regulatory and commercial fact, absent from the clinical setting in which the products are administered. The consequence at the level of the patient is symmetric. The concentrated fetal-derived growth factor and bioactive milieu that supported the *in vitro* signal has no analog in the adult human tissues these products enter. The bench condition cannot exist in the patient, not because clinicians have failed to recreate it, but because the patient's biology does not contain it and cannot be made to contain it.

These two facts together establish that the foundational mechanistic claims for PRP, BMAC, CaHA, and PLLA are irreproducible by design. A clinician who wishes to verify, in their own hands, that PRP release stimulates chondrocyte matrix synthesis as the foundational literature reports cannot do so — they cannot obtain the reagent, and even if they could, the patient tissue into which they inject the product does not provide the conditions the reagent supports. The mechanistic claim is unverifiable not because the relevant trials have not yet been done, but because the conditions under which the claim was generated are structurally unavailable to the persons clinically responsible for the patients on whom the products act. There are, however, two clinical readouts—one from each field examined in this review—that can be used to evaluate the proposed biological mechanism. Unlike subjective outcome measures, these readouts reflect structural and surgical properties of the tissue itself, assessed in living patient's years after treatment.

In the aesthetic field, the readout is the surgeon's operative plane. A deep-plane facelift is, in effect, a direct *in vivo* inspection of the tissue that CaHA and PLLA were claimed to regenerate. If the proposed products produced the organized, native-architecture nucleogenesis that their marketing asserts, the surgeon entering a biostimulator-treated face would find healthy, well-organized tissue gliding along its natural planes. Yet, the reported experience is the opposite. Roughly half of the responding surgeons reported that prior repetitive panfacial filler increased facelift difficulty, while reversible HA was relatively unproblematic [47]. The product the surgeons did not blame is the one that can be dissolved before they operate. This is the foreign-body fibrosis reappearing under the scalpel: what was sold as regeneration presents, at the only inspection that cannot be confounded by the conditions of its own production, as a scar.

In the orthopedic field, the readout is structure and the hard clinical endpoint of arthroplasty. The regenerative claim for PRP and BMAC predicts disease modification: preservation of cartilage and, downstream, the delay or avoidance of joint replacement. After more than two decades and several hundred randomized trials, the corpus has overwhelmingly powered itself on subjective symptom scores measured at 6-12 months, and has only rarely pre-specified the structural or arthroplasty endpoints capable of testing the proposed mechanism. That choice of endpoint is itself an instance of the downstream attribution failure- the field measured what could register an effect without ever distinguishing the proposed mechanism from placebo, from the mechanical and anti-inflammatory effects of the injection, or from regression to the mean.

In the face, the surgeon's dissection plane exposes nucleogenesis as foreign-body fibrosis. In the knee, the structural endpoint exposes cartilage regeneration as indistinguishable from saline. In each, the measurable event is real — the collagen is deposited, the symptom does improve — and in each it is the name that is laundered, the conversion of a repair or non-specific response into a clean regenerative claim. The hard clinical readout that the

mechanism cannot launder — the plane the scalpel cannot find, the cartilage volume that does not change, the arthroplasty that is not avoided — is exactly where the renaming is caught. Clinical reality is the reproducibility test that these mechanisms were never built to pass. It is the test this review insists upon, because it is the only test conducted in the patient, by the persons responsible for the patient, on the tissue the claim is about.

The orthobiologic and aesthetic injectable industries are large, growing, and commercially consequential. The clinical populations exposed to these products number in the millions annually in the United States alone. The cost of off-label and self-pay regenerative and biostimulatory injectables is borne primarily by patients, often outside insurance coverage, on the strength of the mechanistic claims that this review documents as inadequately verified. The professional and commercial incentives to leave the foundational claims unexamined are substantial. The patients exposed to the products have no such incentive.

The aforementioned proposed translational standards are not novel. They are the standards applied to clinical-grade cell therapy manufacturing, to pharmaceutical mechanism-of-action claims, and in our 2025 work to the case for IND-exempt RCT access for lyophilized WJ [9]. The standards are attainable and have already been achieved. The question this review puts to the test is whether they will be required of the products that have not yet had to meet them.

The federal regulatory framework that admitted PRP, BMAC, CaHA, and PLLA under classifications based on inherited mechanistic warrants is not, in the near term, likely to revisit those classifications. The clinical use of these products is too entrenched, the commercial interests are too consolidated, and the institutional capacity for retrospective re-examination is limited. The locus of meaningful regulatory innovation in regenerative medicine has shifted to the states.

State legislatures across the country are presently drafting and enacting bills that expand provider latitude to administer unapproved drugs, stem cell products, and regenerative biologics outside the conventional FDA approval pathway. The legislative impulse behind these bills is patient access and provider autonomy. The methodological obligation that should accompany the legislative impulse has, in the bills enacted to date, been largely absent.

This review proposes that state regulatory frameworks expanding access to unapproved regenerative and stem cell products should, as a condition of that expansion, require comparative IRB-approved trials capable of generating clinically actionable and reproducible evidence. The principle is symmetric and internally consistent. A state that grants providers latitude to administer products outside the federal approval framework assumes responsibility for ensuring that what providers actually do under that latitude is methodologically interrogable. The instrument

by which that responsibility is discharged is the IRB-approved comparative trial — not the case series, not the registry, not the post-marketing surveillance database, all of which are subject to the same attribution failures documented in this review.

10. Conclusion

We propose three specific legislative provisions. First, any state law authorizing provider use of unapproved regenerative biologics or stem cell products should require that products administered under such authorization be enrolled in IRB-approved comparative trials within a defined window, with comparators selected to address clinically meaningful questions. Second, the mechanistic claims supporting any product administered under such authorization should be subject to the three-part translational standard proposed in this review, with state-level review authority to require additional evidence where the foundational literature is documented as inadequate. Third, state IRBs reviewing such trials should be empowered to accept reliance agreements with OHRP-registered IRBs of record for multi-site studies, including international sites, to enable the comparative scale necessary for meaningful evidence generation.

The model is operationally available. IRCM-2025-468 (KOA-001) is being conducted under exactly this structure: an OHRP-registered IRB of record, comparative head-to-head design, pragmatic outcomes appropriate to the clinical question, and international and United States sites under a single protocol. The legislative innovation we propose is that this structure become the expected condition of state-authorized regenerative practice, not the exception.

The deeper alignment is between two propositions that the states are presently in a position to enforce, and the federal framework cannot. The first proposition is that patients should have access to regenerative therapies that the federal pathway has not approved. The second is that the products administered under that access should be subject to the methodological standards that the federal pathway should have required, but did not. State legislatures considering bills that advance the first proposition without addressing the second are creating a market for irreproducible-by-design claims. Burgeoning state regulatory frameworks around which new Stem Cell and Regenerative Therapy Laws are drafted should therefore anticipate and invite both comparative IRB-approved research and expanded clinical use as paired conditions of the same legislative authority.

This is where mechanistic laundering ceases to be an abstraction about citation chains and becomes a fact about patient care. The clinician inherits the laundered claim and cannot make it true at the bedside, because the conditions that generated it were never present in the patient to begin with. The aesthetic surgeon labors through scar to recover a contour the injectable promised to provide. The orthopedic patient undergoes course after course of an injectable sold as cartilage-restoring and arrives at the operating room for the replacement it was meant to forestall. These are the

clinical signatures of a field whose foundational mechanisms were asserted in vitro and never confirmed in vivo by the persons responsible for the result. They are also the reason the corrective this review proposes is not academic: the comparative, mechanism-testing, hard-endpoint trial is not a methodological nicety but the only instrument that can tell a patient, before they pay for and receive one of these products, whether the mechanism on the label is operating in their tissue or only in the literature. Until that instrument is required, the patient remains the place where the unverified claim is finally tested — and, too often, where it is finally found wanting.

Declarations

Conflict of Interest

The author is a physician and researcher affiliated with entities engaged in regenerative medicine clinical practice, research, and product development, and serves as Principal Investigator of IRCM-2025-468 (KOA-001). The author has a research interest in perinatal-tissue biologics, including lyophilized Wharton's Jelly, which is discussed in this review as a methodological counterexample. No product manufacturer or commercial sponsor funded, reviewed, or influenced the design, content, or conclusions of this work. The translational standards proposed herein are applied symmetrically to all product classes discussed, including those in which the author has a research interest.

Ethical Approval (if applicable)

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of the Institute of Regenerative and Cellular Medicine (protocol code IRCM-2025-468 (KOA-001), approved on May 6th, 2026).

AI Usage Statement

Generative AI tools were not used in the writing or editing process of this manuscript.

References

- Obana, K. K., Schallamo, M. S., Saltzman, B. M., et al. (2021). Current trends in orthobiologics: An 11-year review of the orthopaedic literature. *The American Journal of Sports Medicine*, 50, 3121–3129.
- Stein, A. (2007). Decreasing variability in your cell culture. *BioTechniques*, 43, 228–229.
- Van der Valk, J., Bieback, K., Buta, C., et al. (2018). Fetal bovine serum (FBS): Past–present–future. *ALTEX*, 35(1), 99–118.
- Ghebos, R. E., Hui, L., & Wolfram, J. (2025). Increasing the biomolecular relevance of cell culture practice. *Journal of Biomedical Science*, 32, 1–10.
- Weber, T., Malakpour-Permlid, A., Chary, A., et al. (2025). Fetal bovine serum: How to leave it behind in the pursuit of more reliable science. *Frontiers in Toxicology*, 7, Article 1612903.
- Noh, K. C., Liu, X. N., Zhaun, Z., et al. (2018). Leukocyte-poor platelet-rich plasma-derived growth factors enhance human fibroblast proliferation in vitro. *Clinics in Orthopedic Surgery*, 10(2), 240–247.
- Lee, H. W., Choi, K. H., Kim, J. Y., et al. (2020). Proteomic classification and identification of proteins related to tissue healing of platelet-rich plasma. *Clinics in Orthopedic Surgery*, 12(1), 120–129.
- Park, Y. B., Kim, J. H., Ha, C. W., et al. (2021). Clinical efficacy of platelet-rich plasma injection and its association with growth factors in the treatment of mild to moderate knee osteoarthritis: A randomized double-blind controlled clinical trial as compared with hyaluronic acid. *The American Journal of Sports Medicine*, 49(2), 487–496.
- Martin, S. M. (2025). Wharton's jelly in regenerative joint therapy: A case for IND-exempt inclusion in randomized controlled trials. *Journal of Biomedical Research & Environmental Sciences*, 6, 1123–1129.
- Guiotto, M., Raffoul, W., Hart, A. M., et al. (2020). Human platelet lysate to substitute fetal bovine serum in hMSC expansion for translational applications: A systematic review. *Journal of Translational Medicine*, 18.
- Brunner, D., Frank, J., Appl, H., et al. (2010). Serum-free cell culture: The serum-free media interactive online database. *ALTEX*, 27(1), 53–62.
- Van der Valk, J., Brunner, D., De Smet, K., et al. (2010). Optimization of chemically defined cell culture media: Replacing fetal bovine serum in mammalian in vitro methods. *Toxicology in Vitro*, 24(4), 1053–1063.
- James, A. W. (2013). Review of signaling pathways governing MSC osteogenic and adipogenic differentiation. *Scientifica*, 2013, Article 684736.
- Amiri, M., Mecani, R., Niehot, C. D., et al. (2023). Skin regeneration-related mechanisms of calcium hydroxylapatite (CaHA): A systematic review. *Frontiers in Medicine*, 10, Article 1195934.
- Filardo, G., Previtali, D., Napoli, F., et al. (2020). PRP injections for the treatment of knee osteoarthritis: A meta-analysis of randomized controlled trials. *Cartilage*, 13, 364–375.
- Gonzales, V. K., de Mulder, E. L. W., de Boer, T., et al. (2013). Platelet-rich plasma can replace fetal bovine serum in human meniscus cell cultures. *Tissue Engineering Part C: Methods*, 19(11), 892–899.
- Astori, G., Amati, E., Bambi, F., et al. (2016). Platelet lysate as a substitute for animal serum for the ex-vivo expansion of mesenchymal stem/stromal cells: Present and future. *Stem Cell Research & Therapy*, 7.
- Heathman, T. R. J., Glyn, V. A. M., Picken, A., et al. (2015). Expansion, harvest and cryopreservation of human mesenchymal stem cells in a serum-free microcarrier process. *Biotechnology and Bioengineering*, 112(8), 1696–1707.
- Marx, R. E. (2004). Platelet-rich plasma: Evidence to support its use. *Journal of Oral and Maxillofacial Surgery*, 62(4), 489–496.
- Anitua, E. (1999). Plasma rich in growth factors: Preliminary results of use in the preparation of future sites for implants.

21. Aakeda, K., An, H. S., Okuma, M., et al. (2006). Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis. *Osteoarthritis and Cartilage*, 14(12), 1272–1280.
22. Mishra, A., & Pavelko, T. (2006). Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *The American Journal of Sports Medicine*, 34(11), 1774–1778.
23. Anitua, E., Andia, I., Sanchez, M., et al. (2005). Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. *Journal of Orthopaedic Research*, 23(2), 281–286.
24. Lee, D. Y., Lee, S. Y., Yun, S. H., et al. (2022). Review of the current research on fetal bovine serum and the development of cultured meat. *Food Science of Animal Resources*, 42(5), 775–799.
25. Choi, Y. C., Morris, G. M., & Sokoloff, L. (1980). Effect of platelet lysate on growth and sulfated glycosaminoglycan synthesis in articular chondrocyte cultures. *Arthritis & Rheumatism*, 23(2), 220–224.
26. Estevez, B., & Du, X. (2017). New concepts and mechanisms of platelet activation signaling. *Physiology*, 32(3), 162–177.
27. Shi, G., & Morrell, C. N. (2011). Platelets as initiators and mediators of inflammation at the vessel wall. *Thrombosis Research*, 127(5), 387–390.
28. Ngarmukos, S., Tanavalee, C., Amarase, C., et al. (2021). Two or four injections of platelet-rich plasma for osteoarthritic knee did not change synovial biomarkers but similarly improved clinical outcomes. *Scientific Reports*, 11, Article 23603.
29. Bennell, K. L., Paterson, K. L., Metcalf, B. R., et al. (2021). Effect of intra-articular platelet-rich plasma vs placebo injection on pain and medial tibial cartilage volume in patients with knee osteoarthritis. *JAMA*, 326(20), 2021–2030.
30. Qiao, X., Yan, L., Feng, Y., et al. (2023). Efficacy and safety of corticosteroids, hyaluronic acid, and PRP and combination therapy for knee osteoarthritis: A systematic review and network meta-analysis. *BMC Musculoskeletal Disorders*, 24, Article 926. (a) Bannuru, R. R., Osani, M. C., Vaysbrot, E. E., et al. (2019). OARSJ guidelines for the non-surgical management of knee, hip, and polyarticular osteoarthritis. *Osteoarthritis and Cartilage*, 27(11), 1578–1589.
31. Cotter, E. J., Wang, K. C., Yanke, A. B., et al. (2017). Bone marrow aspirate concentrate for cartilage defects of the knee: From bench to bedside evidence. *Cartilage*, 9(2), 161–170.
32. Pittenger, M. F., Mackay, A. M., Beck, S. C., et al. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science*, 284(5411), 143–147.
33. Hernigou, P., Homma, Y., Lachaniette, C. H. F., et al. (2013). Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells. *International Orthopaedics*, 37(11), 2279–2287.
34. Santolini, M., Rios, J. L., Custers, R. J. H., et al. (2025). Mesenchymal stromal cell injections for osteoarthritis: In vitro mechanisms of action and clinical evidence. *The Knee*, 56, 267–275.
35. Chahla, G., Dean, C. S., Moatshe, G., et al. (2016). Concentrated bone marrow aspirate for the treatment of chondral injuries and osteoarthritis of the knee: A systematic review of outcomes. *Orthopaedic Journal of Sports Medicine*, 4(1), Article 2325967115625481.
36. Keeling, L. E., Belk, J. W., Kraeutler, M. J., et al. (2021). Bone marrow aspirate concentrate for the treatment of knee osteoarthritis: A systematic review. *The American Journal of Sports Medicine*, 50(8), 2315–2323.
37. Kim, G. B., Seo, M. S., Park, W. T., et al. (2020). Bone marrow aspirate concentrate: Its uses in osteoarthritis. *International Journal of Molecular Sciences*, 21(9), Article 3224.
38. Yutskovskaya, Y., Kogan, E., & Leshunov, E. (2014). A randomized, split-face, histomorphologic study comparing a volumetric calcium hydroxylapatite and a hyaluronic acid-based dermal filler. *Journal of Drugs in Dermatology*, 13(9), 1047–1052.
39. Stein, P., Vitavska, O., Kind, P., et al. (2015). The biological basis for poly-L-lactic acid-induced augmentation. *Journal of Dermatological Science*, 78(1), 26–33.
40. Nowag, B., Casabona, G., Kippenberger, S., et al. (2022). Calcium hydroxylapatite microspheres activate fibroblasts through direct contact to stimulate neocollagenesis. *Journal of Cosmetic Dermatology*, 22(2), 426–432.
41. Ray, S., & Ta, H. T. (2020). Investigating the effect of biomaterials such as poly-(L-lactic acid) particles on collagen synthesis in vitro: Method is matter. *Journal of Functional Biomaterials*, 11(3), Article 51.
42. Li, X., Li, N., Wang, Y., et al. (2024). Research progress of fibroblasts in human diseases. *Biomolecules*, 14(11), Article 1478.
43. Anderson, J. M., Rodriguez, A., & Chang, D. T. (2008). Foreign body reaction to biomaterials. *Seminars in Immunology*, 20(2), 86–100.
44. Shoulders, M. D., & Raines, R. T. (2009). Collagen structure and stability. *Annual Review of Biochemistry*, 78, 929–958.
45. Ouyang, R., Su, X., Liang, Y., et al. (2025). *Advances in poly-L-lactic acid injections for facial and neck rejuvenation. Plastic and Reconstructive Surgery–Global Open*, 13(5), e7029.
46. Aguilera, S. B., McCarthy, A., Khalifian, S., et al. (2023). The role of calcium hydroxylapatite (Radiesse) as a regenerative aesthetic treatment: A narrative review. *Aesthetic Surgery Journal*, 43(10), 1063–1090.
47. Sweis, I., DeRoss, L., Raman, S., et al. (2023). Potential effects of repetitive panfacial filler injections on facelift surgery and surgical outcomes: Survey results of the members of The Aesthetic Society. *Aesthetic Surgery Journal Open Forum*, 5, ojad010.
48. Wollina, U., & Goldman, A. (2024). Spontaneous and induced degradation of dermal fillers: A review. *Journal of Cutaneous and Aesthetic Surgery*, 17(4), 273–281.
49. Guo, J., Fang, W., & Wang, F. (2023). Injectable fillers: Current status, physicochemical properties, function mechanism, and

-
- perspectives. *RSC Advances*, 13, 23841–23858.
50. Tonnard, P., Verpaele, A., & Romero, A. (2025). Clarification of the concept of “regeneration” in the context of calcium hydroxylapatite and its promotion in regenerative aesthetics. *Aesthetic Surgery Journal*, 46(1), NP5–NP7.
51. Previtali, D., Merli, G., Frattura, G. D. L., et al. (2020). The long-lasting effects of “placebo injections” in knee osteoarthritis: A meta-analysis. *Cartilage*, 13, 185–196.

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