

Dear Reviewer,

We would like to thank you for the time and effort invested in the evaluation of our manuscript.

We acknowledge that Reviewer 1 raises a number of points that are, on a technical level, relevant and that have helped us identify areas where the manuscript can be improved. We have therefore taken into account several of these remarks in the revised version, particularly regarding structure, clarity, and emphasis on tissue-engineering approaches.

However, the general tone complicates constructive exchange, coupled with insinuations about author bias, conflicts of interest, and scientific intent. In our view, such language undermines the perceived impartiality of the evaluation and does not align with the standards of a fair and constructive peer review. It is the reason why, we have decided not to directly respond to the reviewer 1 but instead to the editor.

Please find the answer to the comment of reviewer 1 below.

Comment 1`The title of the paper addresses an important topic in orthopedic tissue engineering. However, the manuscript requires substantial improvements as it has significant issues with organization, analytical depth, critical evaluation, methodological rigor, and technical clarity. Most fundamentally, the title misrepresents the content. The paper purports to focus on “tissue engineering” of the ACL, yet large portions of the text are devoted to general knee anatomy, ACL biomechanics, and conventional surgical treatments, essentially reproducing textbook material. As a result, the title is overly broad and somewhat misleading. In scientific writing, titles should accurately reflect the aim and scope of the work. Here, a more specific title or a narrowed focus is warranted. For example, if the intent is to review only tissue-engineering approaches to ACL repair, the introduction and title should clearly state that narrower focus. Currently, the mismatch between title and content confuses the reader about the paper’s true scope.

Answer: We have modified the title of the review to reflect better the content of the manuscript: The title is now: Anterior Cruciate Ligament Tissue Engineering: Biological Principles, Engineered Substitutes, and Preclinical Outcomes

Comment 2: The organization and focus of the review are also problematic. A high-quality literature review should be organized around clear themes or questions, not simply a sequence of disjointed topics. The manuscript’s structure is diffuse: Sections on basic anatomy, collagen biology, and clinical reconstruction occupy many pages with little connection to the core theme of tissue engineering. In a well-structured review, the

introduction would explicitly outline the review's question and scope, for example, which aspects of ACL tissue engineering are being surveyed, and would identify gaps in current knowledge. Instead, the introduction largely describes ACL injury prevalence and standard grafts, then briefly mentions tissue engineering. The boundaries of the review are not clearly defined, and readers cannot discern a specific organizing principle. As one guide notes, reviewers should check that the "title and approach of the work" are closely aligned and that the authors have a clear research question for their narrative. This paper lacks such framing: the transition from general background to tissue engineering is abrupt, and later sections (especially on artificial ligaments and artificial intelligence) feel like loosely connected appendices rather than integral parts of a coherent review.

Answer: As mentioned in the text, we think the description of anatomy, physiology and pathophysiology are required to reconstruct an organ, what is described just after these sections. We do not think it is a lack of focus. Especially Bioengineering (Basel) published recently a review titled: "Integrating Modern Technologies into Traditional Anterior Cruciate Ligament Tissue Engineering". In this review, the authors states: "An understanding of the anatomy and physiology of the ACL is crucial to construct a synthetic ACL graft using tissue engineering." ... "In order to perform a successful ACL reconstruction, it is beneficial to comprehend the biomechanics of the ACL inside the knee joint." Etc.. We completely agree with this philosophy and firmly believe that understanding an organ is the key to reconstruct it.

Comment 3: In terms of content, much of the material is superficial and lacks novelty. The anatomy and biomechanics sections mostly repeat well-known facts (e.g. knee joint structure, ACL collagen composition) without adding new insights or critical synthesis. Good reviews should synthesize existing literature to advance understanding, rather than simply recount common knowledge. Here, basic information is presented in encyclopedic detail (with numerous older references), but little critical perspective is offered. By contrast, a review of tissue engineering ought to emphasize recent studies, technologies, and remaining challenges in ACL repair. The core tissue-engineering section (Section 4) outlines various biomaterials and techniques (synthetic, natural, hybrid scaffolds, decellularization, cells), but its depth is uneven. Some subsections read like lists of materials with minimal discussion of how each specifically advances ACL repair. Cross-references to ACL context are sparse. For example, it is unclear how the many mentioned biomaterials have performed in ACL-specific experiments. The narrative fails to tie these subtopics together into

a focused discussion. In fact, it reads as if the authors describe general soft-tissue engineering concepts rather than ACL-specific strategies. Thus the section lacks thematic continuity and fails to highlight what is novel or particularly promising about ACL tissue engineering.

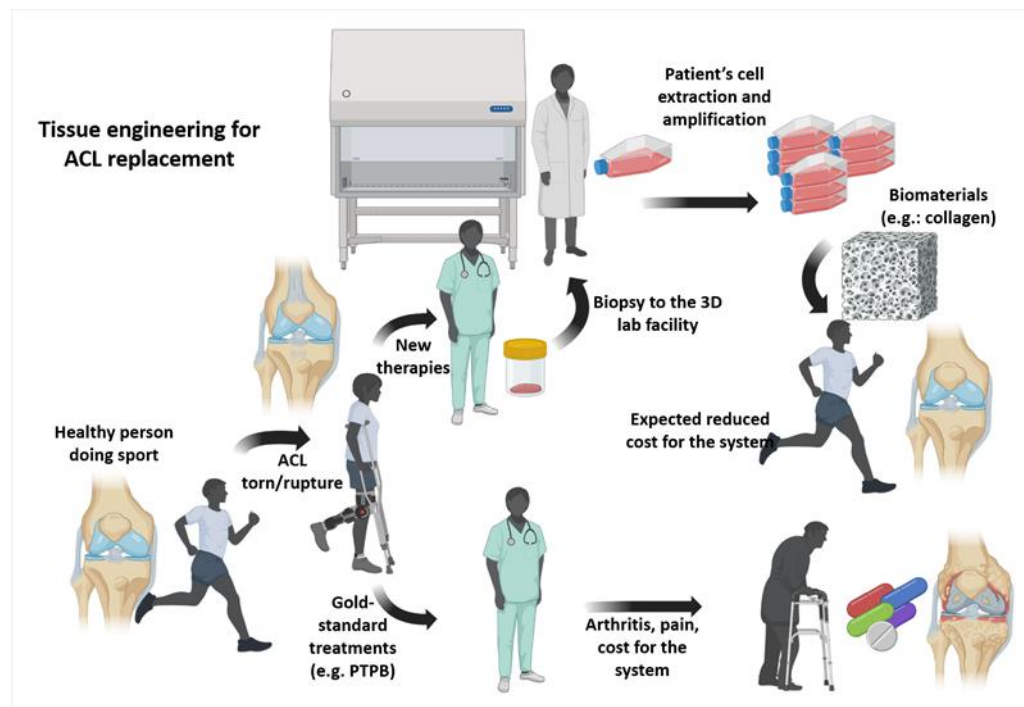
Answer: We add the following text in the introduction section: Most existing reviews on ACL reconstruction focus either on the anatomical and clinical aspects of ligament injury or, conversely, on the biomaterials and engineering strategies designed to address it, but rarely bridge these two dimensions. In contrast, the present review integrates detailed biological, molecular, and biomechanical foundations of the native ACL with a comprehensive assessment of current tis-sue-engineering approaches. By explicitly linking clinical limitations to the structural and biochemical organization of the ligament, this work aims to clarify how fund-mental biology should guide the design of next-generation engineered grafts. This integrative perspective provides a unified framework that is essential for developing therapeutically relevant and physiologically informed strategies for ACL regeneration.

We aknowledge that most of the data will be known by experts but in the case, readers are not experts, they could be useful and avoid them to search these data elsewhere.

Comment 4: The figures and illustrations are also problematic. Figure 1 (knee anatomy) is said to be created in BioRender, but in style and execution it does not appear to match typical BioRender outputs. Regardless, it simply shows basic ligaments without any labeling of magnitudes or references; as drawn, it adds no information beyond what the text already states. Figures in scientific papers must be informative and precise. They should be understandable on their own, with captions that describe all elements and highlight the main message. In this paper, the figure captions are often too brief or vague. For example, Figure 3's caption merely lists "patient-derived cells, culture media, scaffold techniques" in generic terms. An effective caption should "describe everything in the graph, draw attention to its important features, and (when practical) describe the main conclusions to be drawn from it". None of the figures meets this standard. Figure 2 ("Tissue engineering workflow") is especially disappointing. It is visually cluttered and includes non-scientific elements (the reviewer notes a label like "Healthy and happy patient doing sport"), which is inappropriate in a professional manuscript. Flowcharts in reviews should use clear schematic symbols and standardized scientific language. Adding cartoonish or subjective phrases undermines the paper's credibility. The caption for Figure 2 is not self-contained and fails to explain key steps; readers must guess what the icons mean. Moreover, Figures 4 and 5 (reused from the authors' prior

work, see below) include histological images but lack scale bars or calibration. In microscopy and tissue images, including scale bars is essential so that readers can interpret sizes and dimensions. As one source emphasizes, “scales give the reader the key for aligning the image content with reality”. Without scale bars or detailed legends, the histology panels in Figures 4–5 cannot be properly understood or compared. The figures in this manuscript do little to support the discussion; in some cases they merely duplicate known results (or even earlier publications by the same authors) without new interpretation, which per guidelines would be considered redundant or “confirmatory” data that adds little new understanding.

Answer: Explanations about figures were given in the section for reviewers 2 and 3. Figure 2 was modified (see answer to reviewer 3). Figure 4 and 5 come from a published work. The reviewer is fully entitled not to agree with the authors’ graphical choices; however, it would also be appropriate to acknowledge that personal preferences in visual representation are not necessarily universal or superior to alternative approaches.<



Comment 5: The most striking issue is the dominant presence of Dr. Francine Goulet and the Goulet group's work throughout the manuscript. The entire Section 5 ("Example of a successfully goat-implanted bioartificial anterior cruciate ligament") is essentially a detailed exposition of the Goulet group's research spanning decades. This creates several problematic

patterns: the paper reads less as a balanced review and more as a technical report promoting one research program; Section 5 is disproportionately detailed about fabrication steps and staging of integration; and the extensive acknowledgment stating Dr. Goulet "chose not to be listed as an author" despite being the focus of an entire section raises questions about authorship transparency and conflicts of interest.

Answer: We thank the reviewer for raising this point. We understand the concern regarding the balance between the different examples presented in the manuscript. In the revised version, Section 5 has been substantially restructured to ensure that the manuscript reads as a broad and balanced review of the field. The content related to the work of Dr. Goulet's group has been reduced and repositioned so that it now serves only as an illustrative example among other approaches, rather than as the central focus of the review. References to additional studies from the same group have also been removed to avoid any unintended overemphasis.

Regarding authorship, we wish to clarify that Dr. Goulet was invited at the outset to contribute to this review but ultimately declined authorship. In accordance with COPE and MDPI authorship guidelines, an individual who does not participate in writing or revising the manuscript and who requests not to be listed as an author should not appear as such. A brief acknowledgment was therefore included to recognize her long-standing contributions to the field, without implying authorship or involvement in this particular manuscript. We confirm that there is no conflict of interest and that authorship attribution fully complies with journal policies. We hope this clarification is helpful, and we trust that the revised manuscript now addresses the reviewer's concerns while maintaining the scientific integrity and balanced perspective expected from a review article.

Comment 6: Several important topics receive insufficient or no adequate treatment: immune response and graft rejection are mentioned but underdeveloped; mechanical testing standards and what constitutes clinically meaningful performance lack discussion; vascularization and revascularization kinetics are glossed over; proprioceptive/sensory nerve integration is addressed in anatomy but never integrated into tissue engineering strategies; regulatory pathways and clinical trial considerations are nearly absent; cost-effectiveness and manufacturing scalability receive no attention; and long-term in vivo outcomes beyond 12 months are rarely discussed.

Answer: As mentioned by the reviewer himself/herself, these points are briefly discussed. It was not the focus of the review.

Comment 7: Section 7 feels like an add-on rather than an integral part of tissue engineering discussion. Most AI applications discussed (surgical planning, tunnel placement, kinematics prediction) apply to conventional ACL reconstruction rather than bioengineered substitutes. Missing is discussion of AI applications directly relevant to tissue engineering: computational modeling of scaffold design, optimization of degradation kinetics, prediction of integration outcomes, or personalized graft design. The section reads more like a literature scan than critical analysis.

Answer: This section was improved and the text modified as below:

8. Artificial intelligence to improve ACL reconstruction

Artificial intelligence (AI) is a rapidly developing field whose applications are expanding across many domains, particularly in medicine [169]. Machine learning (ML) enhances the accuracy of predictive models through algorithms capable of recognizing and learning reproducible patterns in large datasets [170,171]. Unsurprisingly, AI-based tools now support systematic literature searches with improved efficiency and reproducibility in medical research [170,171]. Deep learning (DL) architectures, including artificial neural networks, are increasingly employed in robotics to execute highly precise surgical procedures [172,173]. Another emerging application is the design of microrobots for advanced drug-delivery strategies, enabling targeted penetration of otherwise inaccessible tissues such as solid tumors [174].

Several ML models have demonstrated impressive accuracy in predicting postoperative complications [175]. In orthopedics, force platforms coupled with transducers or sensors are widely used to assess joint loading, ground-reaction forces, balance, and gait, although their reliability is often limited by the low fidelity of many sensors [176]. Recent DL-based advances now offer new possibilities for non-invasive estimation of joint forces and injury risk during sports or occupational activities [176].

In the context of knee injuries, DL may eventually support the automated assessment of multiple parameters essential for successful ACL reconstruction and long-term stability, including femoral and tibial tunnel positioning, tunnel length, concomitant meniscal lesions, hip-knee-ankle alignment, and tibial slope [10]. DL-based models may also be applied

preoperatively to evaluate knee kinematics as a predictor of post-traumatic osteoarthritis [172]. More broadly, AI-based tools are being developed for fracture diagnosis and classification, risk assessment and outcome prediction, intraoperative navigation, robot-assisted surgery, and even pain-management strategies [182–188].

More recently, ML-based predictive models have been applied specifically to ACL reconstruction. A 2025 systematic review including more than 125,000 patients showed that ML algorithms designed to predict revision ACLR or secondary knee injury achieve strong discriminatory performance (AUC 0.77–0.997), although calibration remains inconsistent across studies [189]. Such models may help identify high-risk patients preoperatively and refine individualized graft-selection and rehabilitation strategies.

AI has also shown potential to enhance postoperative monitoring and rehabilitation. Telerehabilitation systems incorporating “smart” AI-enabled braces have demonstrated functional outcomes comparable—or in some cases superior—to standard in-clinic rehabilitation programs after ACL reconstruction, especially during periods where hospital access is limited [177].

AI-derived models may also support return-to-play decision-making by integrating metrics such as limb symmetry, neuromuscular control, psychological readiness, and movement-quality indicators [180,181].

Finally, recent work suggests that large language models (LLMs) may become useful clinical decision-support tools.

A 2025 study comparing Gemini and ChatGPT-4 demonstrated that Gemini produced responses with greater clarity, completeness, and clinical alignment when evaluated against AAOS guidelines for ACL reconstruction [190]. Such tools could ultimately assist surgeons in synthesizing evidence, planning interventions, and improving patient counselling.

Collectively, these advances indicate that AI may improve postoperative knee stability, optimize graft placement, support functional assessment, and assist orthopedic surgeons in addressing the intrinsic limitations of ACL healing and postoperative rehabilitation [177–181,189,190] (Figure 6).

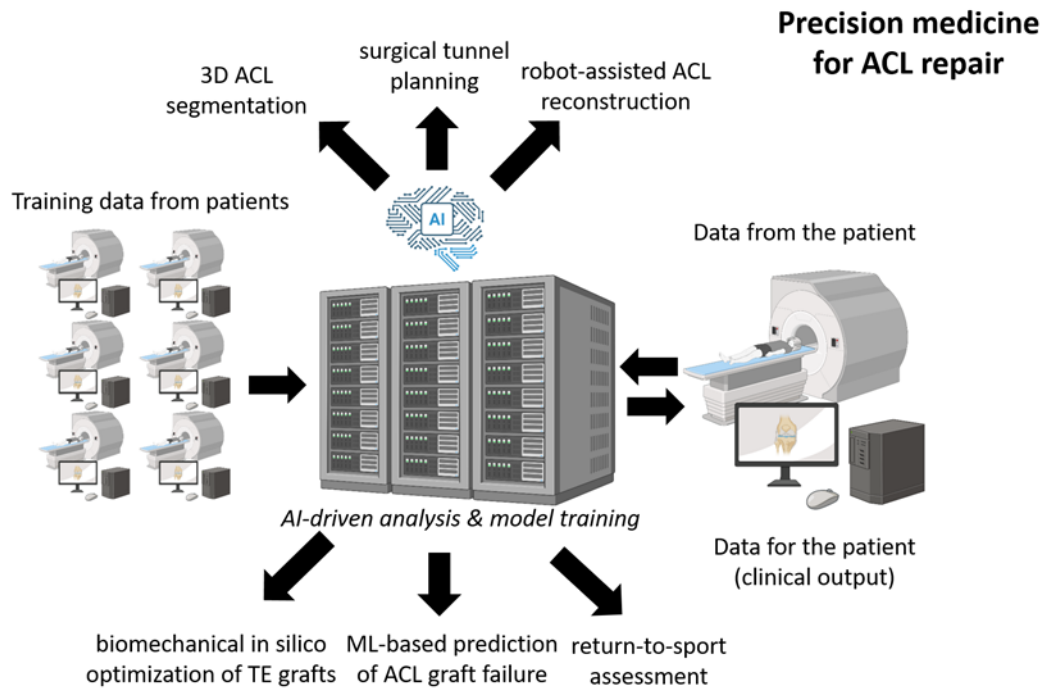


Figure 6. Artificial-intelligence-enabled precision medicine workflow for ACL reconstruction. Large multimodal datasets (imaging, biomechanical recordings, clinical variables) collected from patients are used to train AI models for 3D ACL segmentation, surgical tunnel-planning optimization, robot-assisted ACL reconstruction guidance, in silico biomechanical optimization of tissue-engineered grafts, machine-learning-based prediction of ACL graft failure, and objective return-to-sport assessment. These tools collectively support individualized decision-making and may enhance surgical precision, graft longevity, and functional recovery after ACL repair.

Comment 8: Manufacturing process validation, GMP compliance, FDA/EMA regulatory pathways, scale-up feasibility, cost per implant, sterilization requirements, quality control, and commercial viability are largely absent, yet these factors are as critical as biological function for clinical translation.

Answer: It was not the focus of the review. We think that before to GMP production and commercialization, a safe and efficient product should be proposed.

Comment 9: The Conclusions section is brief and generic, restating problems without offering specific recommendations about which approaches warrant investment or clear guidance on experimental priorities. No timeline prediction for clinical translation is provided.

Answer: the conclusion has been modified as below:

9. Conclusions and perspectives

Anterior cruciate ligament injuries remain a major clinical challenge, particularly in young and physically active individuals. Despite decades of refinement, current re-construction strategies based on autografts or allografts still have significant limitations, including donor-site morbidity, incomplete restoration of native biomechanics, and a high prevalence of post-traumatic osteoarthritis. These limitations underscore the ongoing need for alternative solutions capable of sustainably restoring knee function.

Over the past two decades, tissue engineering has emerged as a promising strategy to address these shortcomings. Synthetic and hybrid constructs provide strong initial mechanics but show incomplete remodeling. In contrast, natural and decellularized scaffolds promote richer cellular integration at the expense of early structural strength. Preclinical studies consistently demonstrate that no single material or strategy currently fulfills all the biological and biomechanical requirements of a functional ACL substitute.

The main challenge for successful ACL tissue engineering lies in achieving an optimal balance between mechanical competence, biological receptivity, and controlled degradation kinetics. Scaffold architecture, fiber alignment, interface integration, and host-graft interactions remain essential determinants of long-term success. Furthermore, the translation of promising preclinical results to clinical practice remains limited by regulatory constraints, manufacturing complexity, and the need for long-term in-vivo validation in clinically relevant large-animal models.

Comment 10: One author affiliated with Christie Innomed; no disclosure of intellectual property; no conflicts of interest statement

Answer: Regarding the reviewer's concern about potential conflicts of interest, we wish to clarify that one co-author is currently employed by Christie Innomed; however, this employment is unrelated to the present

manuscript. The author's involvement in this review is based solely on his previous scientific contribution as first author of the article referenced as [23], which led to the invitation to write this review. Christie Innomed had no role in the conception, writing, funding, material support, or any aspect—direct or indirect—of the present manuscript.

We confirm that there is no intellectual property involved, no financial or professional benefit to Christie Innomed, and no relationship between the company and the topic of the review. In accordance with MDPI guidelines, the Conflicts of Interest statement has been maintained and remains accurate: the authors declare no conflicts of interest.

We respectfully wish to clarify that the manuscript was fully written by the authors. As stated in the Methods section, artificial intelligence tools were not used for scientific writing or content generation; Microsoft 365 Copilot was only employed for minor language polishing after the full draft had been completed and under full author supervision.

Given that the other two reviewers found the English appropriate, we believe the concern raised by Reviewer 1 regarding both language quality and alleged AI authorship may stem from a misunderstanding.