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Review

R0, the new grail — how multimodal molecular imaging, mixed reality, and artificial intelligence contribute to 100% effective surgery☆

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ABSTRACT

Achieving microscopically negative margins (R0) remains one of the main challenges in breast-conserving surgery (BCS), directly influencing local control, cosmetic outcome, and the need for re-excision. Despite advances in localization techniques such as seed placement and intraoperative pathology, incomplete resections still occur in up to 20% of cases. Recent technological developments now offer the opportunity to redefine margin assessment through multimodal intraoperative molecular imaging, artificial intelligence (AI), and mixed reality (AR/VR) visualization. Preoperative imaging – spanning magnetic resonance imaging (MRI), ultrasound, and combined positron-emission tomography (PET) / computed tomography (CT) – provides functional information that guides personalized surgical planning. AI-based segmentation and radiomics enable integration of these datasets into three-dimensional models for accurate tumor mapping and prediction of disease spread. Intraoperatively, radioguided surgery (RGS), intraoperative ultrasound, and fluorescence or gamma systems offer real-time navigation, while label-free techniques such as Raman spectroscopy, impedance analysis, and hyperspectral imaging add biochemical insight at the resection margin. AR/VR tools further enhance spatial orientation by fusing preoperative and intraoperative data into interactive 3D environments. After excision, specimen imaging with micro-CT, Cerenkov luminescence, and high-resolution PET/CT provides immediate verification of margin status. Emerging AI algorithms can interpret these multimodal images in real time, supporting rapid intraoperative decision-making. Together, these innovations define a continuum of planning, navigation, and verification that progressively reduces uncertainty around surgical margins. The convergence of molecular imaging, digital visualization, and AI marks a decisive step toward consistently R0, biologically informed, and truly personalized breast-conserving surgery.

Introduction

Breast cancer remains the most frequent malignancy among women in Spain, with more than 35,000 new cases diagnosed annually and a sustained trend toward early detection, supported by screening programs and improved imaging sensitivity. This context has driven the evolution of breast-conserving surgery (BCS) toward increasingly precise, safe, and minimally invasive procedures, where close collaboration among surgery, radiation oncology, radiology, and nuclear medicine has become essential for comprehensive management.

Yet, positive or close margins after BCS remain common and drive re-excision and local recurrence risk. Contemporary reviews estimate reoperation rates of approximately 20% in many series, and involved margins correlate with higher local recurrence [1,2]. Achieving R0 resection, defined as the complete removal of the tumor with microscopically negative margins, is not only the strongest intraoperative surrogate for local control

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and directly impacts cosmetic outcomes, patient anxiety, and healthcare costs associated with repeat interventions [3].

Despite advances in localization techniques such as wire-guided or radioactive seed placement and intraoperative frozen section or specimen radiography, variability in margin assessment and limited visualization of tumor extent remain major challenges. Conventional localization methods entail additional logistical and ergonomic challenges, such as preoperative coordination with radiology, wire displacement, and patient discomfort, limiting their flexibility in modern workflows. Moreover, the intrinsic biological heterogeneity of breast tumors and the lack of reliable intraoperative visualization of microscopic disease still limit the surgeon's ability to consistently achieve R0 resections.

Recent advances — spanning multimodal molecular imaging for preoperative planning, intraoperative visualization through augmented and mixed reality (AR/MR) environments, and artificial intelligence (AI)–driven analysis — have opened a new era in radioguided and molecular image-guided surgery (RGS/MIGS). This emerging concept, sometimes referred to as "molecular surgery," integrates functional imaging and targeted radiopharmaceuticals to visualize tumor biology in real time, going beyond purely anatomical guidance [4,5].

Building on these concepts, this paper presents a concise review of current developments aimed at achieving 100% effective breast-conserving surgery in terms of clean resection margins (R0). We focus on three key domains of innovation:

(a) preoperative imaging and artificial intelligence for surgical planning,
(b) intraoperative navigation and real-time margin control, and
(c) ex-vivo specimen imaging for on-site verification of tumor bed clearance.

We explicitly exclude developments in intraoperative pathology or molecular tissue analysis, such as One-Step Nucleic Acid Amplification (OSNA).

Preoperative imaging as a surgical roadmap

Accurate delineation of tumor extent and its spatial relationship to glandular structures and cosmetic landmarks is the cornerstone for defining adequate resection margins while preserving breast shape and function. Preoperative imaging not only guides localization but also determines the strategy for achieving R0 resections by identifying occult multifocality, intraductal spread, and potential nodal involvement.

Conventional imaging modalities — mammography, ultrasound, and magnetic resonance imaging (MRI) — provide complementary anatomical and morphological information, enabling reliable estimation of tumor size and localization [6]. However, anatomic imaging alone may underestimate disease extent or fail to distinguish viable tumor tissue from post-biopsy or treatment-related changes. To address these limitations, molecular imaging using positron-emission tomography (PET) with 18F-fluorodeoxyglucose (FDG) has expanded preoperative assessment beyond anatomy. New tracers such as 18F-fluoroestradiol (18F-FES), 68Ga-FAPI, or HER2-targeted agents provide insight into receptor status and tumor microenvironment, highlighting biologically active regions relevant for margin definition and personalized surgical planning [7–9].

AI, particularly automated segmentation and multimodal data fusion, is now further enhancing preoperative workflows. Deep-learning models can integrate MRI, PET/computed tomography (CT), and ultrasound data to generate accurate, reproducible 3D tumor reconstructions and functional maps, reducing workload for radiologists and nuclear medicine specialists while providing surgeons with intuitive visualizations for operative planning [10]. These outputs form the basis for augmented and virtual reality (AR/VR) surgical rehearsal environments introduced later in this review (Fig. 1).

Beyond visualization, AI enables the extraction of quantitative imaging descriptors — so-called radiomic features — that correlate imaging phenotypes with histopathological and genomic parameters. Such image-derived biomarkers can predict lymph node involvement, molecular subtype, or risk of complications before surgery, thus informing both the surgical plan and the extent of resection [11,12]. Integrating these radiomic and radiogenomic data with molecular or transcriptomic profiles may ultimately refine patient-specific surgical strategies.

Intraoperative navigation and margin assessment

Information derived from preoperative molecular imaging and AI-driven analysis can be transferred into the operating room through radioguided and imaging-assisted navigation systems, bridging planning and execution of precise resections.

Radioguided surgery (RGS) has evolved markedly since its introduction as an alternative to wire localization for nonpalpable breast lesions. Techniques such as radioguided occult lesion localization (ROLL), sentinel node and occult lesion localization (SNOLL), and radioactive seed localization (RSL) enable simultaneous identification of the lesion and its lymphatic drainage pathways, reducing rates of positive margins and reoperations compared with wire-guided procedures [13]. Magnetic seeds and RFID tags now extend these benefits to non-radioactive workflows, simplifying logistics and eliminating radiation safety concerns while maintaining high localization accuracy.

Among intraoperative imaging modalities, intraoperative ultrasound (ioUS) remains the most widely used technique. ioUS provides real-time anatomical feedback on tumor margins and cavity geometry, supporting targeted excision and optimal cosmetic outcomes, particularly in nonpalpable or irregular lesions. When combined with radioguided or seed/tag-based localization, ioUS provides complementary spatial orientation and improves surgical confidence [14].

Beyond localization, several label-free techniques are being developed for intraoperative evaluation of resection margins. Raman spectroscopy detects biochemical fingerprints of malignancy through inelastic light scattering; handheld Raman probes, especially when combined with AI-driven signal analysis, have shown high accuracy for distinguishing tumor from normal tissue within seconds [15]. Impedance-based systems measure tissue dielectric properties in situ; multiple clinical studies have reported lower positive-margin and re-excision rates compared with conventional inspection [16]. Finally, hyperspectral imaging (HSI) offers a non-contact, wide-field approach capturing spectral signatures of tissue oxygenation and composition. Preliminary studies show sensitivities and specificities above 90% for margin detection, with deep-learning methods under active development for automated classification [17].

In the realm of intraoperative molecular imaging, portable gamma cameras introduced the first imaging capability to RGS, translating point-detection gamma signals into visual maps [18]. They allow intraoperative and ex-vivo confirmation of lesion removal — typically detecting Tc-99 m-labeled primary tumors in ROLL/SNOLL or I-125-seeds in RSL — and can be combined with optical or fluorescence modules for hybrid imaging of functional and superficial anatomy, such as the vasculature [19].

Beyond building on planar systems, freehand SPECT (fhSPECT) extends RGS into 3D, reconstructing tomographic datasets in real time (Fig. 2). Clinical studies confirm its feasibility for simultaneous localization of tumors and sentinel nodes, verification of excised specimens, and evaluation of residual activity without prolonging operative time [20,21]. These systems are clinically deployable with short learning curves and pave the way for routine multimodal intraoperative navigation integrating molecular, optical, and anatomical information.

The increasing integration of these imaging and sensing modalities creates the foundation for AR/VR applications, where preoperative datasets and real-time intraoperative signals can be co-registered and visualized within the surgical field for intuitive 3D navigation.

Mixed reality visualization

Virtual reality (VR) and augmented reality (AR) technologies integrate preoperative and intraoperative imaging into a spatially registered 3D

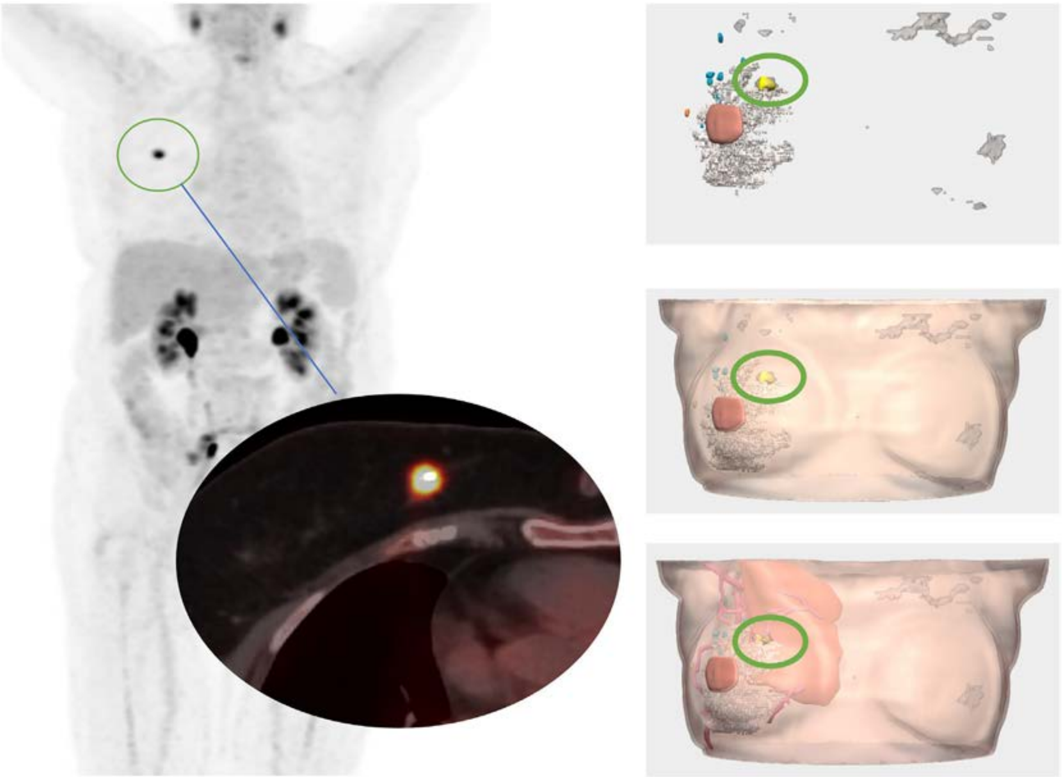

Figure 1. AI-assisted segmentation of a right breast carcinoma using whole-body and prone-position PET/CT. The tumor (highlighted in yellow and circled in green) was segmented using a deep learning algorithm. The VR visualizations illustrate anatomical deformation alignment between supine and prone acquisitions, allowing accurate anatomical correlation and surgical planning through layered visualization of surrounding structures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

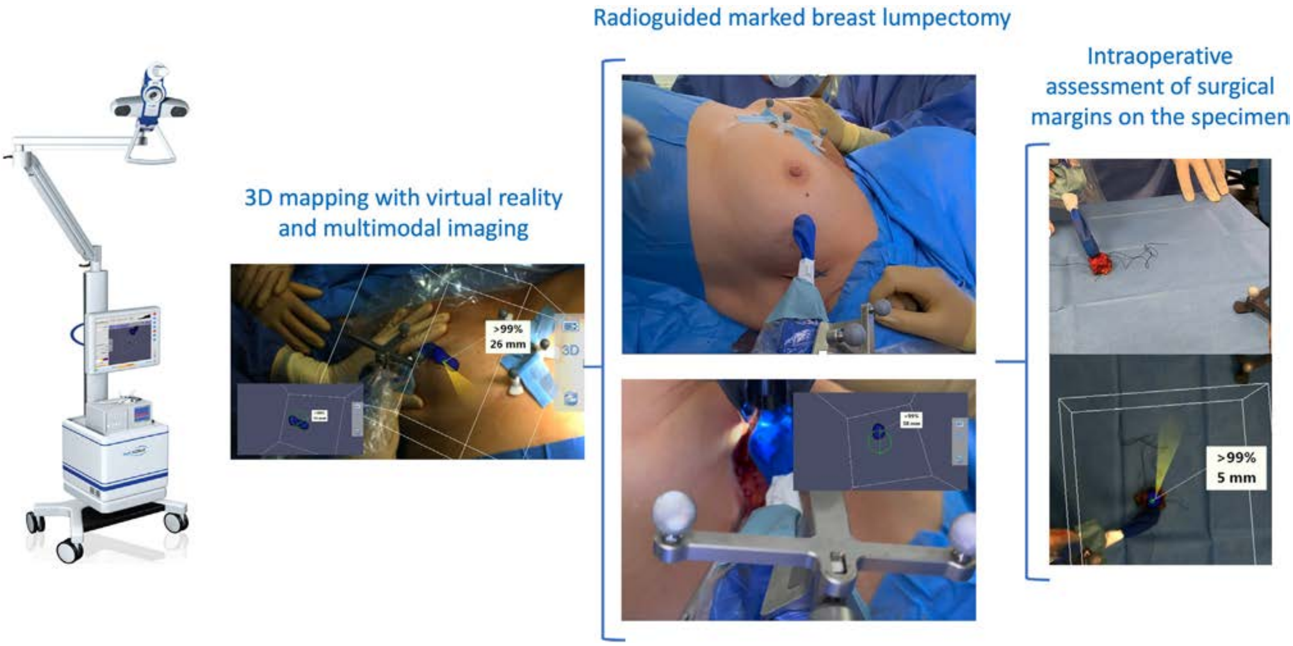

Figure 2. Radioguided I-125 seed-localized breast lumpectomy performed with 3D navigation and multimodal imaging (here combination of optical and fhSPECT). The system provides real-time depth estimation and intraoperative guidance using AR/VR visualization, enabling complex surgical planning and immediate margin assessment of the excised specimen.

environment that can be visualized directly within the operative field. By overlaying segmented datasets from MRI, PET/CT, or single-photon-emission tomography (SPECT) onto the patient's anatomy, AR/VR enables surgeons to intuitively understand tumor extent, lymphatic drainage, and critical structures during dissection [5,22,23].

Early feasibility studies in oncologic and reconstructive surgery have demonstrated that AR/VR can accurately project anatomical landmarks and pre-marked lesions using optical or fiducial registration and sensors placed directly in the operating room. In breast surgery, however, its application remains technically challenging due to soft-tissue deformation and patient positioning differences between imaging and the operating table. Despite these constraints, AR/VR may enhance spatial orientation in complex resections or re-excisions, and serves as a powerful tool for surgical planning, team communication, and education [23,24].

Integration of AR/VR with real-time tracking technologies and intraoperative updates from ioUS, gamma cameras, or fhSPECT could allow dynamic correction of image overlays and improved registration accuracy. When coupled with AI-based segmentation and radiomics, AR/VR systems could evolve into adaptive “surgical cockpits” that integrate functional, molecular, and anatomical data for personalized intraoperative navigation [5,23].

Clinical validation of AR/VR in breast-conserving surgery is still limited to small feasibility studies, and its quantitative impact on margin status or operative outcomes remains to be demonstrated. Nonetheless, AR/VR represents a crucial step toward unifying multimodal information streams into a single, intuitive visual platform.

These developments naturally converge with advances in specimen imaging, where the same multimodal datasets can be used to verify resection completeness and assess margins immediately after excision.

Specimen imaging and on-site margin evaluation

Following tumor excision, ex-vivo specimen imaging provides direct verification of margin status before the patient leaves the operating room. The goal is to identify any residual tumor at the resection edge and guide immediate re-excision when necessary.

Specimen radiography remains the standard intraoperative margin assessment method in most centers. It offers rapid feedback on lesion localization, clips, and calcifications, but its performance decreases in dense tissue or lesions without radiographic contrast, leading to possible false negatives [13]. Micro-computed tomography (micro-CT) enables three-dimensional evaluation of excised tissue with sub-millimeter resolution and good correlation with histopathology [25].

Cerenkov luminescence imaging (CLI) detects optical photons emitted by β-particles from radiotracers such as 18F-FDG, visualizing metabolic activity on the specimen surface within minutes. Its sensitivity is high, but depth penetration is limited, making it best suited as a complementary surface-imaging tool [26]. To overcome the depth limitations of CLI, high-resolution specimen PET/CT extends intraoperative assessment from structural to functional imaging (Fig. 3). The excised tissue can be scanned immediately in the operating room using compact mobile devices [27]. Follow-up studies demonstrated accurate visualization of residual metabolic activity near margins, with sensitivities and specificities around 85–90%, clearly outperforming specimen radiography [28].

Automated analysis may further accelerate specimen evaluation. For example, deep-learning segmentation models have achieved margin-status predictions comparable to expert readers, suggesting the feasibility of AI-assisted decision support for real-time PET/CT interpretation [29].

Discussion and outlook: toward consistently R0 surgery

The persistent challenge of achieving microscopically negative margins (R0) has driven decades of innovation in BCS. The technologies described in this review — molecular imaging, AI, intraoperative navigation, and AR/VR — represent converging paths toward the same objective: to visualize, understand, and control the true biological extent of disease at the moment of resection.

Preoperative imaging now provides a 3D biological map of the tumor, enabling surgeons to plan resections based not only on morphology but also on metabolic and receptor-defined boundaries. Intraoperatively, radioguided and optical tools translate this information into actionable guidance, while label-free and molecular imaging methods verify margins

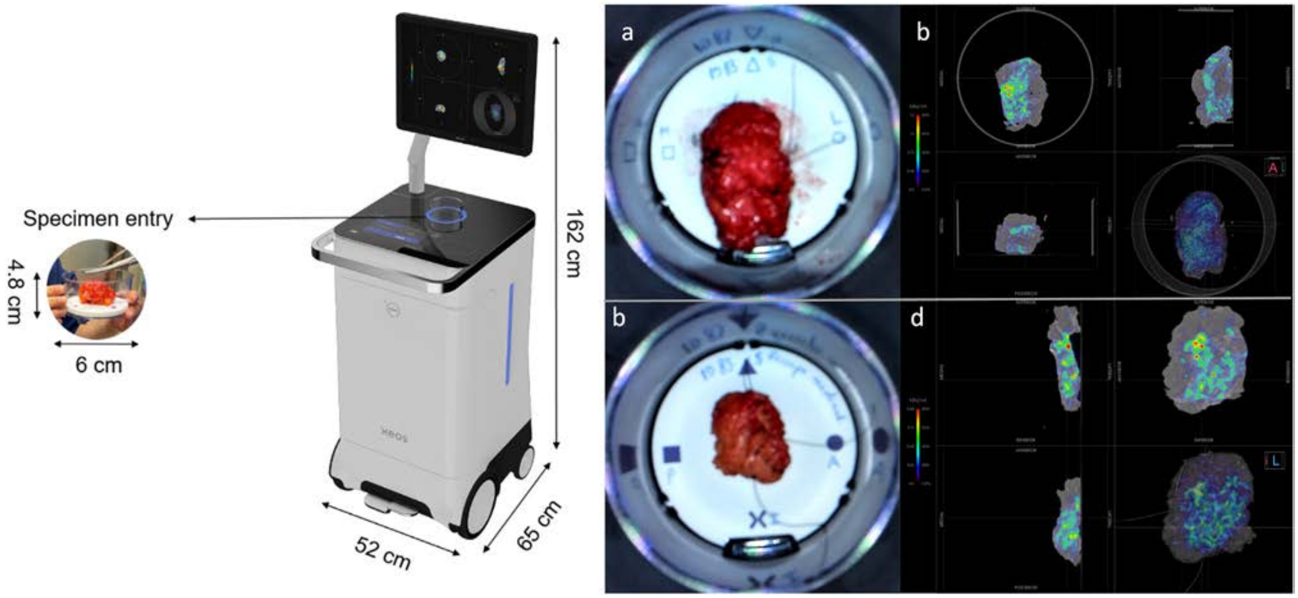

Figure 3. Specimen imaging using high-resolution specimen PET/CT: Left – Device showing specimen tray. Right – Exemplary case: (a) optical image of primary tumor specimen; (b) FDG PET/CT image of primary specimen showing metabolic activity at the medial border suggestive of a R1 resection status, which was the case on histopathology; (c) optical image of additional resected tissue at medial side of primary tumor bed following review of the surgeon of the PET/CT scan of the primary specimen in the operation room; (d) FDG PET/CT image of additional resected tissue showing metabolic activity at the supected side of the specimen but with negative final margins, confirmed as a final R0 resection on histopathology and hence a second surgery was avoided.

in real time. Post-excision, specimen imaging and AI-assisted interpretation close the feedback loop, validating the completeness of resection before the wound is closed.

Together, these developments outline an integrated, data-driven workflow — a continuum of planning, navigation, and verification — that progressively reduces uncertainty around margin status.

In this emerging paradigm, intraoperative molecular imaging often serves as a functional vector, linking tumor biology to surgical decision-making. Its signal, interpreted through advanced imaging and analytics, allows margins to be defined by biological activity rather than arbitrary geometric distances. This shift from anatomical to molecular precision redefines what “complete resection” means and moves breast surgery closer to the ideal of a 100% effective, patient-tailored procedure.

However, achieving consistent R0 outcomes through these technologies will require more than technical refinement. Standardization of imaging protocols, real-time data integration across modalities, and clinical validation in multicentric trials are essential to translate feasibility into impact. Similarly, usability, cost, and logistical factors remain key gatekeepers to widespread implementation. The goal is not to replace surgical judgment but to augment it with objective, multimodal information, ensuring that every decision in the operating room is informed by the most complete representation of the disease.

Ultimately, the path toward R0 breast-conserving surgery embodies the broader evolution of surgical oncology: from visual estimation to evidence-guided precision, from static images to dynamic, intelligent navigation, and from reactive correction to proactive margin control. As these tools mature, the definition of success in breast surgery will extend beyond clear margins to encompass functional preservation, esthetic outcome, and molecular completeness — realizing the promise of truly personalized, data-driven cancer surgery.

Conclusion

The convergence of multimodal molecular imaging, mixed reality visualization, and artificial intelligence is fundamentally transforming the paradigm of breast-conserving surgery from anatomical estimation to biological precision. By establishing a continuous information pipeline—from preoperative metabolic mapping and AI-driven planning through intraoperative radioguided navigation and real-time margin assessment to immediate ex-vivo verification—these technologies collectively address the persistent challenge of positive margins that has limited BCS outcomes for decades. While individual modalities have demonstrated feasibility and promising performance metrics, the true potential lies in their systematic integration into unified, adaptive workflows that translate tumor biology into actionable surgical guidance at every decision point.

Realizing the vision of consistently R0-effective surgery will require moving beyond isolated technical validation toward multicentric clinical trials that assess integrated platforms in real-world settings, alongside efforts to standardize protocols, optimize cost-effectiveness, and ensure seamless intraoperative usability. The ultimate objective is not to replace clinical judgment but to augment it with objective, multimodal data streams that reduce uncertainty and enable surgeons to perform truly personalized resections—where margins are defined by molecular boundaries rather than arbitrary distances, and where completeness, safety, and cosmesis are simultaneously optimized. As these technologies mature and converge, the aspiration of 100% effective breast-conserving surgery transitions from theoretical ideal to achievable clinical standard, embodying the broader evolution of surgical oncology toward data-driven, biologically informed precision.

Informed consent

Not applicable.

Ethical considerations

Not applicable.

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Use of artificial intelligence

Internet searches were partially supported by AI-assisted tools (Google, Perplexity). Language was improved using ChatGPT.

Authors' contributions

TW drafted an outline of the review, contributed to all sections, integrated input from BL and JO, reviewed all references and completed the final version.

JO contributed to the introduction, preoperative imaging, intraoperative imaging, mixed reality and discussion, created Figs. 1 and 2 and reviewed the final version.

BL contributed to specimen imaging and discussion, created Fig. 3 and reviewed the final version.

Conflict of interest

None.

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