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E12 Sheet Plastination of Sus scrofa domestica Temporomandibular Joint: Integrating CBCT and MRI for Enhanced Anatomical Visualization

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SUMMARY: Accurate visualization of the temporomandibular joint (TMJ) is critical for comparative anatomical studies, surgical training, and biomechanical research. This study demonstrates the combined use of cone beam computed tomography (CBCT), magnetic resonance imaging (MRI), and E12 sheet plastination to elucidate the morphology of the porcine TMJ. Fresh TMJ samples were harvested from domestic pigs (Sus scrofa domestica) immediately post-mortem and scanned using CBCT to capture high-resolution images of osseous structures. MRI was subsequently employed to visualize soft tissues, including the articular disc and surrounding ligaments, enabling pre-plastination correlation of bony and soft tissue relationships. Following imaging, the specimens were frozen, serially sectioned into 2–3 mm sheets, and dehydrated through a graded series of acetone through freeze substitution, then impregnated under vacuum with E12 epoxy resin. Finally, the slices were cured. The integrated approach yielded three complementary datasets: (1) CBCT images clearly delineating cortical and trabecular bone architecture, (2) MRI scans highlighting cartilage and synovial structures, and(3) durable, anatomically faithful E12 plastinated slices suitable for direct macroscopic inspection. Correlation of the pre-plastination imaging with the plastinated slices validated both the fidelity of the plastination process and the utility of multimodality imaging. The E12 sheets provided transparent, thin slices that preserved key features of the TMJ, including the articular surfaces, disc, ligaments, and joint capsule, facilitating comparative and functional analyses. This combination of CBCT, MRI, and E12 sheet plastination offers a powerful, integrative method to study the complex anatomy of the porcine TMJ. By fusing high-resolution radiographic data with tangible, anatomically precise plastinated sections, researchers and educators gain comprehensive insights into TMJ morphology, enabling enhanced comparative anatomical research, surgical planning, and teaching applications.

KEY WORDS: E12 sheet plastination, Temporomandibular joint, Porcine model, Cone beam computed tomography, Magnetic resonance imaging.

INTRODUCTION

The temporomandibular joint (TMJ) is an anatomically complex structure that combines osseous, cartilaginous, ligamentous, and synovial components whose coordinated function is essential for mastication, swallowing, and speech (Bordoni & Varacallo, 2023). A comprehensive understanding of its three-dimensional architecture and topographic relationships is crucial not only for comparative anatomy and morphological research but also for surgical training and clinical planning in maxillofacial surgery and orthodontics (Lovasova *et al*., 2018; Iwanaga *et al*., 2019).

While modern imaging technologies have transformed the study of joint anatomy, they still face inherent limitations in representing all components of the TMJ in a correlated and spatially integrated manner. Cone beam computed tomography (CBCT) offers high-resolution visualization of osseous structures with minimal radiation exposure, whereas magnetic resonance imaging (MRI) enables the depiction of soft tissues such as the articular disc, joint capsule, and ligaments without the use of ionizing radiation. However, both modalities provide indirect representations, and interpreting them requires mental or digital reconstruction of anatomical space (López-Ramírez *et al*., 2024).

In this context, the E12 plastination technique stands out as a powerful anatomical tool, allowing for the creation of thin, transparent, and durable physical sections of anatomical specimens in which both hard and soft tissues are preserved in their original spatial relationships (Ottone, 2023). Developed by von Hagens, the E12 technique uses epoxy resin to impregnate tissue following dehydration through cold acetone substitution and vacuum impregnation. The result is rigid, high-quality anatomical slices suitable for direct visual inspection, making them valuable for both educational and research purposes (Von Hagens *et al*., 1987; Ottone *et al*., 2018; Latorre *et al*., 2019).

The true potential of E12 plastination is revealed when combined with pre-plastination imaging techniques such as CBCT and MRI, allowing for three-dimensional anatomical correlation between radiological data and physically preserved structures. This integration enables cross-validation across modalities and reinforces plastination not only as a preservation technique but as a methodological tool for teaching, research, and surgical simulation (Cook & Al-Ali, 1997; Sora & Cook, 2007; Ottone *et al*., 2018; Ottone, 2023; Toaquiza *et al*., 2024).

In the present study, we implemented a multimodal approach combining CBCT, MRI, and E12 plastination for the morphological analysis of the porcine TMJ, a model widely used for its structural similarities to the human TMJ. Our objective was to demonstrate how E12 plastination, in conjunction with pre-processing imaging, can offer a precise and tangible representation of joint anatomy, enhancing both anatomical research and the development of high-quality educational resources. This strategy not only validates the accuracy of imaging techniques but also bridges the gap between digital anatomy and real anatomical structures, offering new possibilities for the integrated study of complex joints.

MATERIAL AND METHOD

Specimen Collection and Preparation. Five fresh temporomandibular joint (TMJ) specimens were obtained from adult domestic pigs (Sus scrofa domestica), immediately post-mortem, and were preserved at -25°C until processing. Specimens were rinsed in running water to defrost and to remove blood residues. The mandibular condyle, glenoid fossa, and associated soft tissue structures were preserved intact during dissection.

Cone Beam Computed Tomography (CBCT). CBCT imaging was performed prior to any invasive processing. Each TMJ specimen was stabilized in a custom-made holder to ensure anatomical alignment and minimize motion during scanning. Imaging parameters were as follows: voxel size 0.2 mm, 90 kVp, 10 mA, and a field of view (FOV) of 100 ¥ 100 mm. Multiplanar reconstructions (axial, coronal, and sagittal) and three-dimensional (3D) volume renderings were generated using CBCT software to assess osseous landmarks and cortical-trabecular architecture.

Magnetic Resonance Imaging (MRI). After CBCT, specimens were allowed to equilibrate to 4°C and scanned using a 1.5 Tesla MRI system (Siemens) equipped with a dedicated surface coil. The joints were embedded in agar gel to maintain hydration and positional stability. T1-weighted and T2-weighted spin echo sequences were acquired in the sagittal and coronal planes. Imaging parameters included: repetition time (TR) 600 ms (T1) and 3500 ms (T2), echo time (TE) 10 ms (T1) and 80 ms (T2), slice thickness 2 mm, slice gap 0.5 mm, field of view 120 mm, and matrix size 256 ¥ 256. These sequences enabled detailed visualization of the articular disc, retrodiscal tissues, ligaments, and synovial membrane.

Epoxy (E12) Plastination technique. The E12 sheet plastination technique was performed following established protocols previously developed and validated by our group (Ottone *et al*., 2016; Ottone *et al*., 2018a, b; Ottone, 2023).

a. Sectioning and Pre-Plastination Freezing. After imaging, the specimens were initially frozen at –25?°C for one week, followed by an additional 24 hours at –80?°C to preserve tissue integrity prior to mechanical sectioning. Each

temporomandibular joint (TMJ) was then serially sectioned into 2–3?mm slices using a precision band saw cooled with liquid nitrogen. Sectioning was performed with careful anatomical orientation, and each slice was sequentially numbered and digitally photographed to ensure proper serial order.

b. Dehydration via Freeze Substitution. Tissue slices were dehydrated through freeze substitution using 100% acetone. Dehydration was carried out in a graded series of acetone baths at –25?°C over a period of three weeks. Acetone concentration and water displacement were monitored every 3–4 days using an acetonometer, and dehydration was considered complete once stabilization was achieved (acetone concentration ≥99.5%).

c. Forced Impregnation with Epoxy Resin. After dehydration, slices were immersed in a mixture of Biodur® E12 epoxy resin and E6 hardener. Impregnation was carried out under vacuum conditions in a vacuum chamber at 5 mmHg at room temperature. The pressure was gradually reduced over several hours to facilitate the replacement of acetone with resin within tissue interstices. Impregnation was conducted over 24 hours, during which bubbles ceased to emerge from the tissue, indicating saturation.

D. Curing and Finishing. Resin-impregnated slices were positioned between glass plates coated with a release agent and cured in an oven at 50 °C for five days. Following polymerization, excess resin was carefully trimmed. All plastinated slices were subsequently labeled, catalogued, and stored at room temperature for long-term preservation.

RESULTS

CBCT Imaging. CBCT imaging produced high-resolution images of the TMJ's bony components. Clear differentiation of the mandibular condyle, glenoid fossa, and articular eminence was achieved. Cortical thickness and trabecular patterns were readily identifiable, facilitating precise anatomical measurements.

MRI Findings. MRI provided excellent contrast between soft tissues. The articular disc appeared as a biconcave, hypointense structure interposed between the mandibular condyle and temporal bone. Ligaments, including the lateral and posterior attachments, were visualized, and synovial fluid spaces were delineated in T2-weighted sequences.

E12 Sheet Plastination. The plastinated slices retained excellent anatomical fidelity. Structures such as the articular disc, joint capsule, and intra-articular ligaments were preserved and visually accessible. The transparent nature of the E12 sheets allowed for both surface and internal analysis without distortion. Integration of the imaging data with plastinated slices validated the spatial correspondence of anatomical landmarks across modalities.

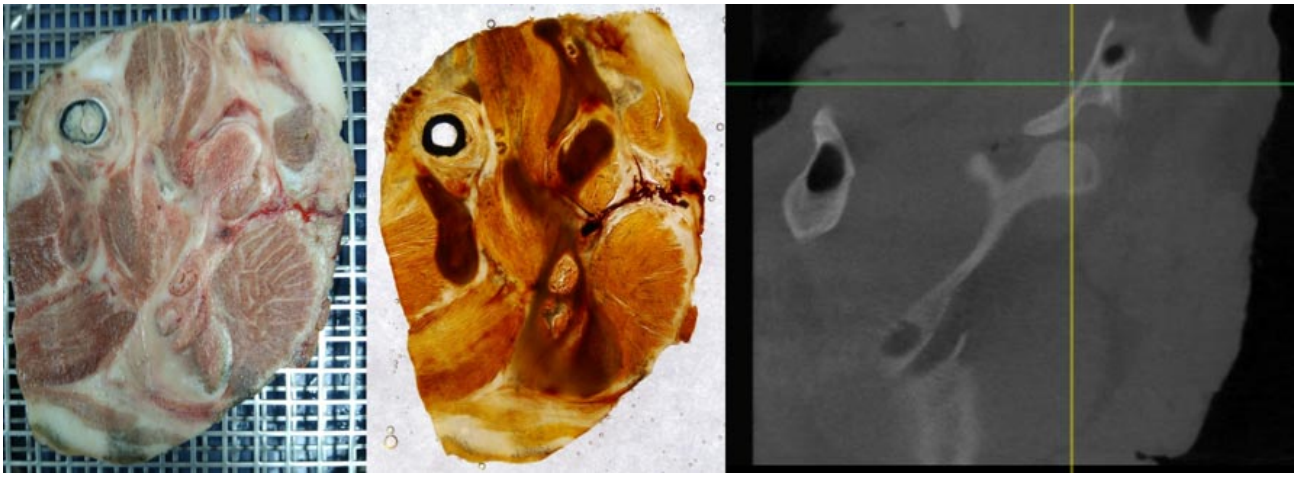

Fig. 1. Multimodal anatomical visualization of the porcine temporomandibular joint (TMJ). A. Fresh coronal section of the porcine TMJ obtained post-mortem, illustrating gross anatomical structures prior to processing. B. Corresponding E12 plastinated sheet of the same specimen, showing preserved morphology of osseous and soft tissue components, including the mandibular condyle, articular disc, and adjacent musculature. C. Cone beam computed tomography (CBCT) image of the same anatomical level, clearly delineating bony contours of the condyle and glenoid fossa. This sequence demonstrates the spatial fidelity and complementary information provided by gross dissection, plastination, and radiological imaging.

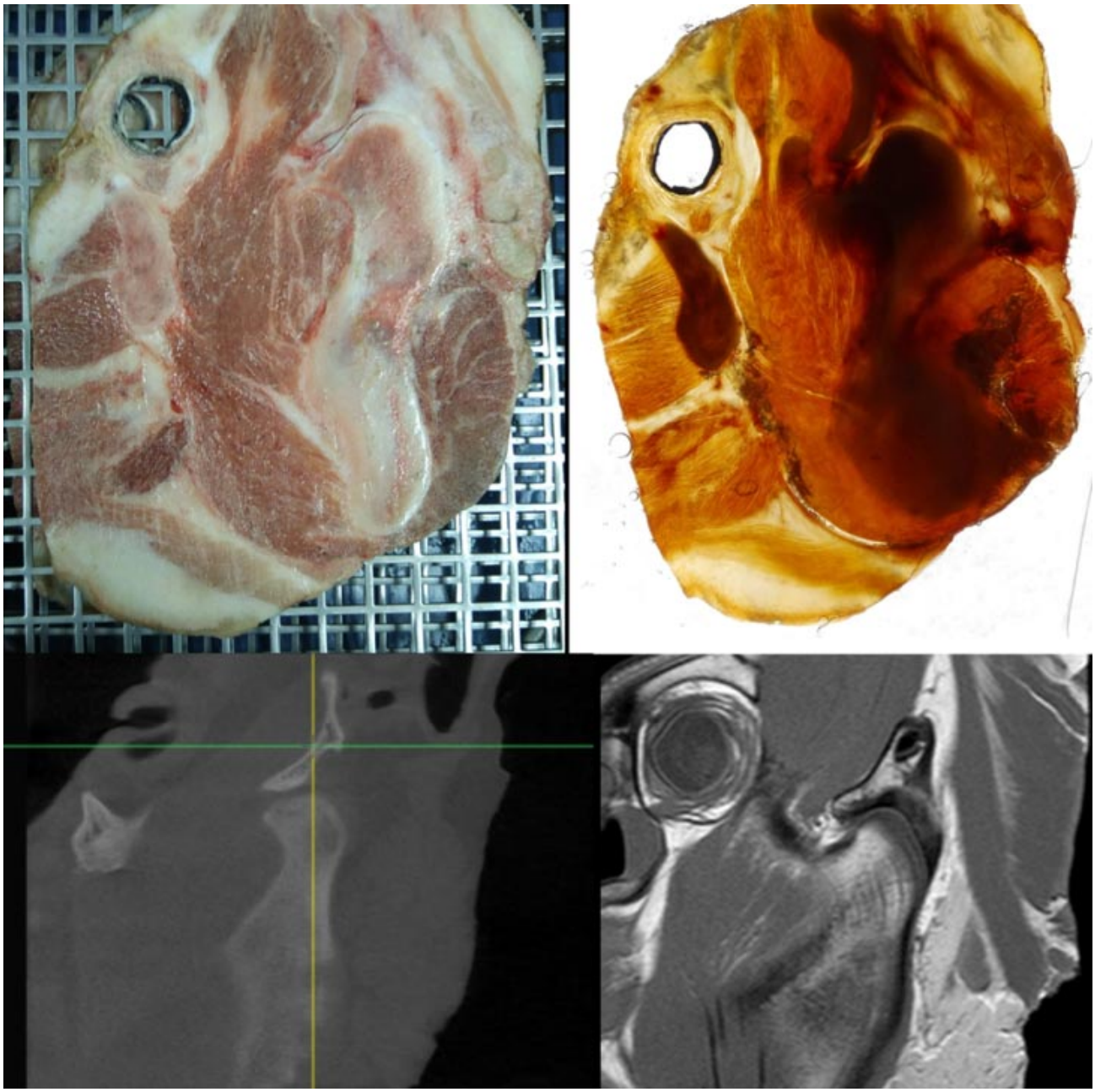

Fig. 2. Comparative multimodal visualization of the porcine temporomandibular joint (TMJ). A. Fresh anatomical coronal section showing the TMJ and surrounding musculature prior to plastination. B. Corresponding E12 plastinated slice, revealing detailed preservation of soft tissues, including the articular disc, joint capsule, and adjacent musculature, as well as the osseous structures of the mandibular condyle and temporal bone. C. Cone beam computed tomography (CBCT) image displaying osseous architecture and alignment of the condylar head within the glenoid fossa. D. Magnetic resonance imaging (MRI) scan of the same region, highlighting the articular disc and soft tissue components of the TMJ. This panel illustrates the high anatomical fidelity and complementary insights provided by E12 plastination and radiological imaging techniques.

DISCUSSION

This study highlights the efficacy of an integrative, multimodal approach, combining CBCT, MRI, and E12 sheet plastination, for the comprehensive anatomical analysis of the porcine temporomandibular joint (TMJ). Among the three modalities employed, E12 plastination emerged as a pivotal technique, enabling not only the preservation of complex joint structures

but also their direct inspection and correlation with pre-plastination imaging. The fusion of high-resolution radiological data with durable, anatomically faithful physical sections contributes to a deeper and more reliable understanding of TMJ morphology and supports the broader application of plastination in research, teaching, and surgical planning.

One of the principal advantages of E12 plastination lies in its capacity to preserve both hard and soft tissues with exceptional spatial accuracy (Pashaei, 2010). While traditional histological techniques provide cellular-level detail, they often distort spatial relationships due to tissue shrinkage, sectioning artifacts, and differential staining (Xu *et al*., 2025). In contrast, plastinated slices maintain the three-dimensional architecture of anatomical structures, allowing the visualization of spatial relationships that are essential for functional and biomechanical interpretations (Steinke, 2001; Thomas *et al*., 2004). In the present study, key elements of the TMJ, such as the articular disc, joint capsule, ligaments, and bone interfaces, were preserved with remarkable clarity and correspondence to their *in vivo* imaging appearance.

The use of CBCT provided highly detailed visualization of the osseous components of the TMJ, including cortical and trabecular bone patterns (Larheim *et al*., 2014). This information was critical for understanding the bony architecture and for guiding the alignment of the subsequent MRI and plastinated data. However, CBCT lacks sensitivity to non-mineralized tissues, highlighting the need for complementary soft tissue imaging (Bede & Tareef Noaman, 2024). MRI, as expected, offered superior contrast resolution for intra-articular structures such as the articular disc, retrodiscal tissues, and synovial components (Bag *et al*., 2014). By capturing pre-plastination images, MRI enabled the identification and mapping of soft tissue landmarks that were later confirmed and visualized in the plastinated slices.

The correlation between pre-plastination imaging and the final plastinated sections was one of the most compelling findings of this study. This cross-validation confirmed that E12 plastination does not significantly distort or displace anatomical elements and can therefore serve as a gold standard for validating radiological interpretations. Moreover, the plastinated slices provided tactile and visual access to anatomical features that may be challenging to appreciate fully through imaging alone, particularly in complex joints like the TMJ, where three-dimensional orientation is critical.

Another notable advantage of E12 plastinated slices is their utility in educational and clinical settings. Unlike radiological data, which often requires specific software and interpretation skills, plastinated specimens offer an intuitive, hands-on learning experience. Their durability, ease of handling, and capacity for long-term storage make them ideal resources for anatomy curricula, surgical workshops, and comparative anatomy studies. In particular, the thin, transparent E12 sheets created in this study can be incorporated into light boxes, digital scanning systems, or augmented reality platforms to further enhance interactive anatomical learning (Soal *et al*., 2010; Ottone *et al*., 2014).

From a comparative anatomy perspective, the porcine TMJ model proved to be highly suitable for exploring mammalian joint architecture. The similarities in joint structure between pigs and humans support the translational relevance of the findings (Almarza *et al*., 2018; Lee *et al*., 2022). By employing this multimodal strategy, future studies may investigate pathological changes in the TMJ, developmental variations, or the outcomes of experimental surgical interventions with greater accuracy and anatomical fidelity.

Despite its strengths, the study has some limitations. The sample size was limited to five specimens, which restricts the statistical generalizability of the findings. Furthermore, while the plastination process preserved most anatomical features effectively, subtle biochemical or histological changes cannot be assessed through E12 plastination alone. Future work could incorporate histological validation, quantitative morphometrics, or digital 3D reconstruction to further enhance anatomical resolution and data integration.

CONCLUSION

This study underscores the powerful role of E12 plastination within a multimodal imaging framework for anatomical research. When combined with CBCT and MRI, plastination bridges the gap between digital imaging and physical anatomy, offering a comprehensive, validated, and pedagogically rich approach to joint analysis. The methodology developed here is not only applicable to the TMJ but also extensible to other complex anatomical regions, advancing the field of anatomical sciences through innovation and integration.

CONFLICT OF INTEREST. The authors declare no conflict of interest.

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