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Decellularized swine dental pulp extracellular matrix: a comparison between formed and developing teeth

Matriz extracelular de polpa dental suína descelularizada: comparação entre dentes formados e em formação

Matriz extracelular de pulpa dental porcina descelularizada: una comparación entre dientes formados y en formación

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ABSTRACT

Objective: To decellularize and characterize, histologically and biochemically, the extracellular matrix (ECM) of dental pulp from fully formed (M1) and developing (M2) swine molars, comparing native and decellularized tissues. **Methods:** Experimental in vitro study using coronal pulps from molars of commercially slaughtered swine, divided into M1 (erupted, with a complete root) and M2 (intraosseous, developing). An enzyme-free, Triton X-100-free protocol based on EDTA and SDS was applied. DNA and collagen were quantified, and histology (H&E, Masson's trichrome and DAPI) and scanning electron microscopy were performed; data were analysed by one-way ANOVA with Tukey's post-hoc test ($p<0.05$). **Results:** Decellularization removed the cellular content of both pulps, with absence of nuclei and DNA reduction of 92.7% in M1 and 96.8% in M2 ($p<0.0001$), with no residual difference between groups ($p=0.60$). M2 pulps, collected from the maxilla only, showed a greater yield per tooth (1.173 g versus 0.377 g) and maintained stable collagen. A porous extracellular matrix scaffold was observed in both groups, with mineralized deposits suggestive of intrinsic mineralization, exclusive to decellularized M2. **Conclusion:** The conservative protocol effectively decellularized both pulps. Teeth at distinct developmental stages provide ECM with particular characteristics, which is relevant to scaffold selection in regenerative endodontics.

Keywords: Extracellular matrix, Regenerative endodontics, Dental pulp, Swine.

RESUMO

Objetivo: Descelularizar e caracterizar, histológica e bioquimicamente, a matriz extracelular (MEC) da polpa de molares suínos formados (M1) e em formação (M2), comparando tecidos nativos e descelularizados. **Métodos:** Estudo experimental in vitro com polpas coronárias de molares suínos de abate comercial, divididas em M1 (irrompidos, com raiz formada) e M2 (intraósseos, em formação). Aplicou-se protocolo livre de enzimas e de Triton X-100, à base de EDTA e SDS. Realizaram-se quantificação de DNA e de colágeno, histologia (H&E, Tricrômico de Masson e DAPI) e microscopia eletrônica de varredura, com ANOVA de uma via e post-hoc de Tukey ($p<0,05$). **Resultados:** A descelularização removeu o conteúdo celular de ambas as polpas, com ausência de núcleos e redução do DNA de 92,7% em M1 e 96,8% em M2 ($p<0,0001$), sem diferença

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residual entre os grupos (p=0,60). As polpas M2, coletadas apenas da maxila, renderam maior massa por dente (1,173 g contra 0,377 g) e mantiveram o colágeno estável. Observou-se arcabouço poroso em ambos os grupos, com incrustações mineralizadas **Conclusão:** O protocolo conservador descelularizou eficazmente ambas as polpas. Dentes em estágios distintos de desenvolvimento fornecem MEC com características próprias, relevantes para a seleção do arcabouço em endodontia regenerativa.

Palavras-chave: Matriz extracelular, Endodontia regenerativa, Polpa dentária, Suínos.

RESUMEN

Objetivo: Descelularizar y caracterizar, histológica y bioquímicamente, la matriz extracelular (MEC) de la pulpa de molares porcinos formados (M1) y en formación (M2), comparando tejidos nativos y descelularizados. **Métodos:** Estudio experimental in vitro con pulpas coronarias de molares porcinos de matadero comercial, divididas en M1 (erupcionados, con raíz formada) y M2 (intraóseos, en formación). Se aplicó un protocolo libre de enzimas y de Triton X-100, a base de EDTA y SDS. Se realizaron cuantificación de ADN y de colágeno, histología (H&E, Tricrómico de Masson y DAPI) y microscopía electrónica de barrido, con ANOVA de una vía y post-hoc de Tukey (p<0,05). **Resultados:** La descelularización eliminó el contenido celular de ambas pulpas, con ausencia de núcleos y reducción del ADN del 92,7% en M1 y del 96,8% en M2 (p<0,0001), sin diferencia residual entre los grupos (p=0,60). Las pulpas M2, recolectadas solo del maxilar, rindieron mayor masa por diente (1,173 g frente a 0,377 g) y mantuvieron el colágeno estable. Se observó un andamio poroso en ambos grupos, con incrustaciones mineralizadas exclusivas de M2 descelularizado. **Conclusión:** El protocolo conservador descelularizó eficazmente ambas pulpas. Los dientes en etapas distintas de desarrollo proporcionan MEC con características propias, relevantes para la selección del andamio en endodoncia regenerativa.

Palabras clave: Matriz extracelular, Endodoncia regenerativa, Pulpa dental, Porcinos.

INTRODUCTION

Tissue engineering depends on scaffolds that provide three-dimensional support to cells and reproduce the microenvironment of the target tissue (ALKSNE M, et al., 2022; REMAGGI G, et al., 2022). Decellularization of native tissues generates extracellular matrix (ECM) scaffolds with reduced immunogenicity (STERN MM, et al., 2009), capable of hosting cells for regenerative medicine, with or without recellularization, and of serving as a basis for hydrogels and bioinks (CRAPO PM, et al., 2011).

The quality of a decellularized scaffold depends on the balance between removing the cellular material, which is responsible for the recipient's immune response, and preserving the three-dimensional architecture together with the structural and signalling components of the matrix, such as collagens, laminins and glycosaminoglycans. Aggressive protocols remove DNA easily but degrade the matrix and compromise cell adhesion and differentiation during recellularization; conservative protocols preserve the matrix better but require objective demonstration that cell removal was effective (CRAPO PM, et al., 2011).

ECM-derived products of animal origin are already used clinically in soft-tissue, bone and cardiac reconstruction. Hydrogels made from natural proteins have attracted interest for their biocompatibility and their capacity to reproduce functional properties of the ECM (DING G, et al., 2015; TREVIZANI M, et al., 2025). Dental pulp, besides being a source of stem cells (WEISS JB, et al., 2020), may serve as a scaffold for tissue engineering; laminin, a natural constituent of the pulp, is a precursor of polylaminin hydrogels with reported regenerative applications in neural lesions (COELHO-SAMPAIO T, et al., 2020).

In endodontic practice, pulp trauma before complete root formation compromises tooth development, resulting in short roots with thin walls and a poorer prognosis. Revitalization and revascularization therapies have been described with success, which makes materials capable of favouring new pulp formation desirable (KHURSHID Z, et al., 2022). Swine dental pulp is widely discarded at slaughter and is therefore an abundant, low-cost source, free from the ethical dilemmas and volume limitations associated with obtaining human pulp. Few dental pulp matrices have been described (ALQAHTANI Q, et al., 2018), there is no gold standard

decellularization protocol (ELNAWAM H, et al., 2024) and studies comparing pulps at different developmental stages are practically non-existent. The developing pulp differs from the mature pulp not only in cellularity but also in the composition and organization of its extracellular matrix, which may translate into scaffolds with distinct structural and biological properties after.

METHODS

This is an experimental in vitro study, descriptive and comparative, with a 2 × 2 factorial design crossing tooth type (M1, erupted permanent molar with a formed root; M2, developing intraosseous molar) and tissue condition (native and decellularized), totalling four experimental groups: native M1, decellularized M1, native M2 and decellularized M2. All stages were carried out at the Universidade Federal de Juiz de Fora (UFJF), except scanning electron microscopy, performed at the Universidade Federal do Espírito Santo (UFES).

Ethical considerations and material sourcing

Fresh swine hemiarches were obtained, under institutional authorization, from certified slaughterhouses in Juiz de Fora-MG, Brazil. The material consisted exclusively of by-products of animals slaughtered as the commercial routine of the establishment, under official sanitary inspection for human consumption, with no breeding, housing or experimental intervention involving live animals for the purposes of this study. Animal Use Ethics Committee (CEUA) review was therefore not required according to the applicable institutional/legal framework, namely Brazilian Law No. 11,794/2008, known as the Arouca Law (MARQUES RG, et al., 2009). The animals were of commercial crossbred Duroc-based genetics, with a mean age of approximately 180 days according to supplier information; their exact genetic composition and chronological age were not recorded.

Pulp collection

The hemimandibles were disarticulated from the maxilla. Teeth were sectioned mesiodistally with a carborundum disc mounted on a straight handpiece, without reaching the pulp; the crown was then detached from the root portion, exposing the coronal pulp, which was collected with surgical forceps and curettes. M1 pulps (erupted permanent molars with a formed root) were obtained from both maxilla and mandible. M2 pulps (developing, intraosseous molars) were obtained from the maxilla only and required removal of the bone plate with forceps and chisel. The material was washed in cold saline solution, stored in 50 mL Falcon tubes and frozen. Pulps from 13 pigs were used, corresponding to 12.5 heads, since only one hemiarch was obtained from one of the animals; pulps were collected randomly from different animals. Two groups were defined: M1 (pulps from erupted, fully formed permanent molars) and M2 (pulps from developing molars impacted in bone).

Decellularization protocol

Incubation was carried out in 0.1% EDTA with 10 mM Tris for 2 h under agitation; three washes in PBS (10 min each); overnight incubation in 0.1% SDS with 10 mM Tris under agitation; a further series of PBS washes (3 × 10 min); and overnight maintenance in PBS with streptomycin. The protocol is free of enzymes (nucleases) and of Triton X-100.

DNA quantification

From approximately 50 mg (dry weight) of each group (n=3), DNA was extracted with lysis solution (10 mM Tris-HCl, 0.1 M EDTA, 0.1% SDS, pH 8.0), maceration in a Tissue Lyser II (Qiagen), proteinase K digestion (55 °C, overnight), phenol/chloroform/isoamyl alcohol extraction (25:24:1) and precipitation with ammonium acetate/ethanol. Integrity was assessed by 1% agarose gel electrophoresis and quantification by spectrophotometry (NanoDrop 2000, 260/280 nm).

Collagen quantification

Collagen content was determined by hydroxyproline assay (n=3), using a modified hydrolysis method: 10 mg (dry weight) hydrolysed in 9 N HCl at 100 °C for 15 h; reaction with chloramine-T and Ehrlich's reagent; reading at 550 nm. Collagen content was calculated considering hydroxyproline as 14.3%, with a standard curve from 0 to 10 µg/mL.

Histological characterization

Paraffin-embedded tissues were sectioned at 3 µm at three depths. For H&E, Harris haematoxylin (40 s) and eosin (1 min) were used. Masson's trichrome followed the standard protocol. For DAPI, 4 µm sections obtained in a cryostat were stained and observed under a fluorescence microscope (Olympus BX51).

Scanning electron microscopy

Two decellularized and two native pulps from each group were analysed (n=8). Fixation was performed in 2.5% glutaraldehyde with 2% formaldehyde in 0.1 M cacodylate buffer; post-fixation in 1% osmium tetroxide with potassium ferrocyanide; dehydration in ethanol; critical point drying; and gold-palladium sputter coating. Observation was carried out using a JEOL JSM-6610LV scanning electron microscope.

Statistical analysis

DNA and collagen data (n=3 per group) were compared by one-way ANOVA followed by Tukey's post-hoc test (HSD) for multiple comparisons among the four groups. The significance level adopted was $p<0.05$. GraphPad Prism software was used for the statistical analysis.

RESULTS

Yield and anatomy

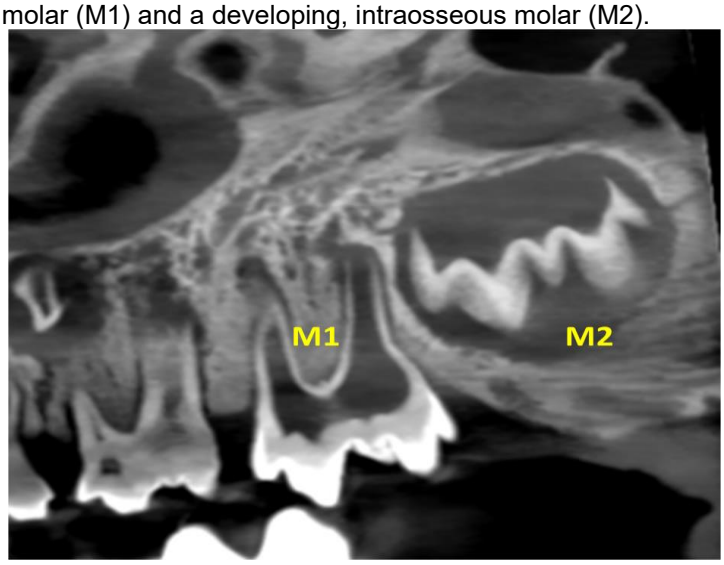
Considering the teeth available for collection in a single piece from slaughter, the mean weight per pulp was 1.173 g in M2 and 0.377 g in M1 — about 211% greater in M2 (**Table 1**). Because M2 teeth were collected from the maxilla only, the number of teeth entered in **Table 1** was adjusted accordingly (25 M2 teeth against 50 M1 teeth for the same 12.5 heads), so that yield is expressed as mean weight per pulp, normalized to the teeth actually collected in each group, and the two groups remain directly comparable on a per-pulp basis; the restriction to maxillary teeth is nevertheless acknowledged as a limitation. Computed tomography confirmed that M2, even without complete crown formation, is larger than M1 in the mesiodistal and buccopalatal dimensions (**Figure 1**).

Table 1 - Native pulp yield per tooth type, by total weight and mean weight per pulp.

Tooth type	Heads / teeth collected	Total weight	Mean weight per pulp
M1	12.5 heads / 50 teeth	18.87 g	0.377 g
M2*	12.5 heads / 25 teeth	29.32 g	1.173 g

*M2 pulps were collected from the maxilla only; the number of teeth used as the denominator was adjusted accordingly, so that the mean weight per pulp remains directly comparable between groups. **Source:** Weiss JB, et al., 2026.

Figure 1 - Computed tomography showing a formed, erupted molar (M1) and a developing, intraosseous molar (M2).



Source: Weiss JB, et al., 2026.

Macroscopically, native pulps from both groups had a reddish colour and a gelatinous consistency. After the protocol, both groups became milky white and translucent, retaining the original shape of the pulp chamber, a change consistent with removal of the cellular and blood content.

DNA quantification

DNA quantification (ng/mg dry weight; n=3; mean ± SEM) differed significantly among groups (one-way ANOVA, p<0.0001; **Figure 2**): native M1 102.95 ± 0.61; decellularized M1 7.56 ± 1.37; native M2 302.76 ± 1.67; and decellularized M2 9.64 ± 0.49.

Decellularization significantly reduced DNA content in both pulp types (Tukey, p<0.0001), by 92.7% in M1 and 96.8% in M2, confirming effective cell removal. In native tissues, M2 showed about 2.9 times more DNA than M1 (p<0.0001), consistent with a younger and more cellularized tissue. After decellularization, M1 and M2 did not differ from each other (7.56 versus 9.64 ng/mg; p=0.60), indicating that, despite very different initial DNA loads, the protocol brought both to the same low residual level.

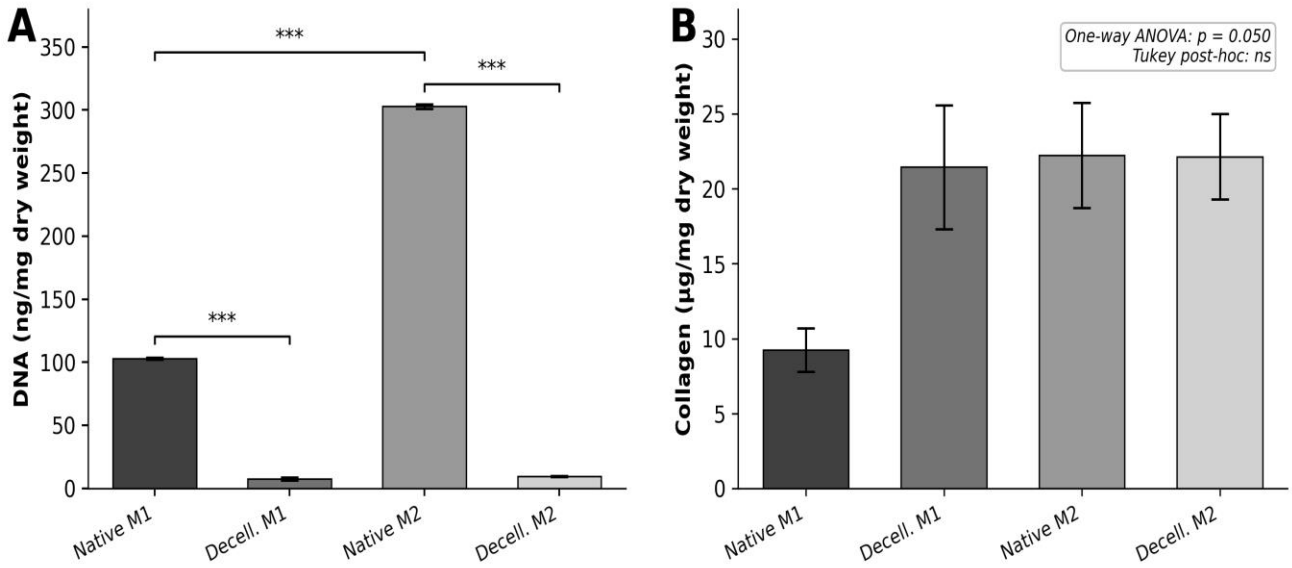
Collagen quantification

Collagen content (µg/mg dry weight; n=3; mean ± SEM) was 9.25 ± 1.45 in the native M1 group, 21.45 ± 4.14 in decellularized M1, 22.24 ± 3.52 in native M2 and 22.15 ± 2.84 in decellularized M2 (**Figure 2**). The one-way ANOVA did not reach the significance level adopted in this study ($F(3,8)=4.08$; $p=0.050$, against $\alpha=0.05$), and Tukey's post-hoc test identified no statistically significant differences among groups (all $p>0.05$). A trend towards increased collagen content was observed in M1 pulps after decellularization (approximately 132%), whereas M2 pulps maintained similar values before and after processing.

Native M2 pulps showed higher mean collagen content than native M1 pulps, although this difference was also not significant.

The increase observed in M1 probably reflects the relative enrichment of the collagenous fraction resulting from removal of cellular components and non-collagenous proteins during decellularization, rather than additional collagen synthesis. These results should therefore be interpreted as a trend, whose confirmation depends on studies with a larger sample size.

Figure 2 - Biochemical quantification of native and decellularized groups (M1 and M2). (A) DNA (ng/mg dry weight): one-way ANOVA with Tukey's post-hoc test; ***p<0.0001; the two decellularized groups did not differ (p=0.60). (B) Collagen (hydroxyproline assay; µg/mg dry weight): one-way ANOVA p=0.050; Tukey's post-hoc test not significant (ns). Data presented as mean ± SEM, n=3.



Source: Weiss JB, et al., 2026.

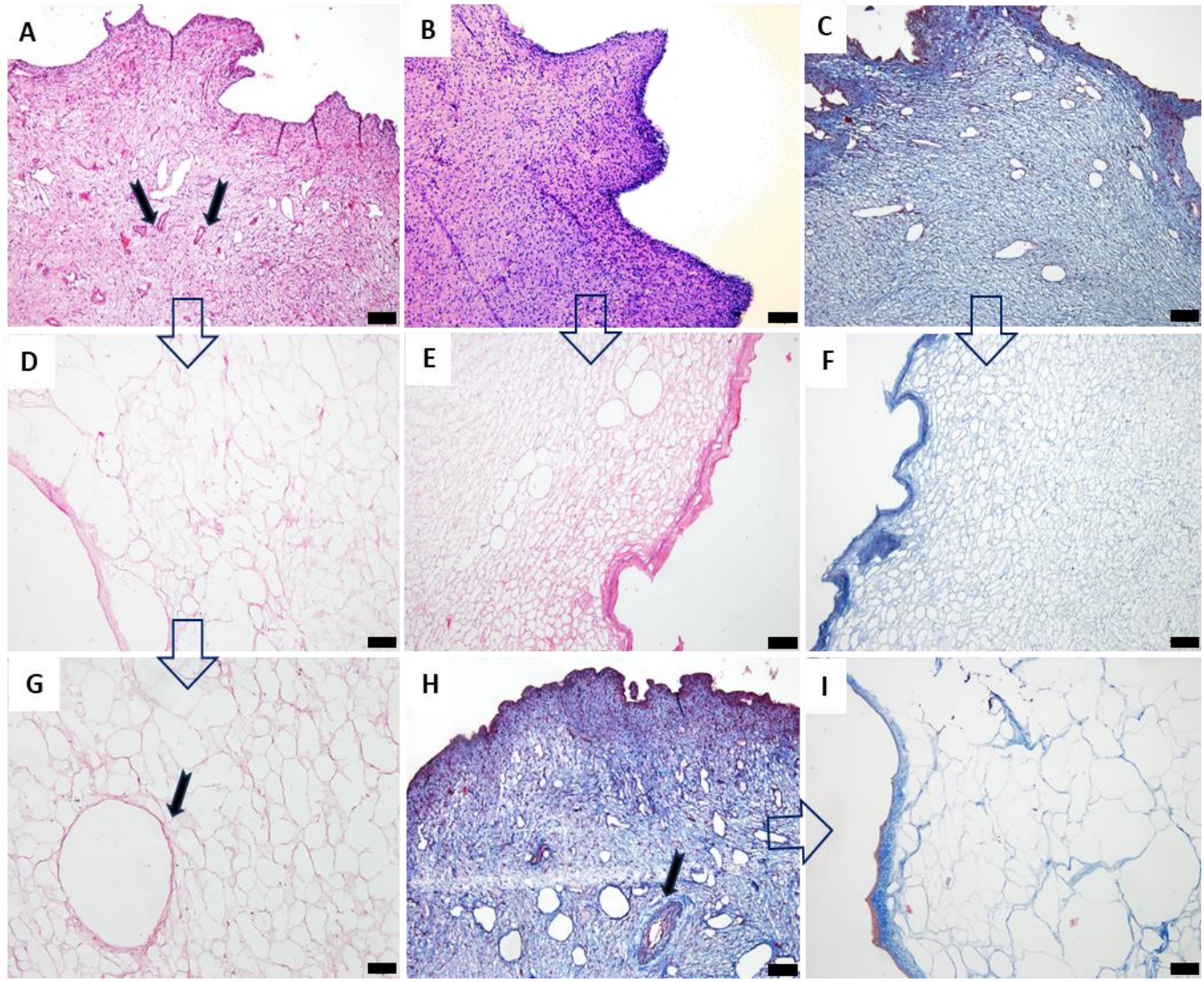
Histology

Histological characterization by H&E and Masson's trichrome is shown in **Figure 3**. Nuclei were abundant in the native panels and minimal to absent in the decellularized panels, corroborating the cell removal also evidenced by DNA quantification and confirmed by DAPI fluorescence (**Figure 4**).

The tissue network was better preserved in decellularized M2. A notable difference was observed in the vascular component between the two developmental stages: native M1 showed distinct blood vessels lined by stained endothelial cells, whereas native M2 showed immature vascular structures, still forming and not lined by endothelial cells.

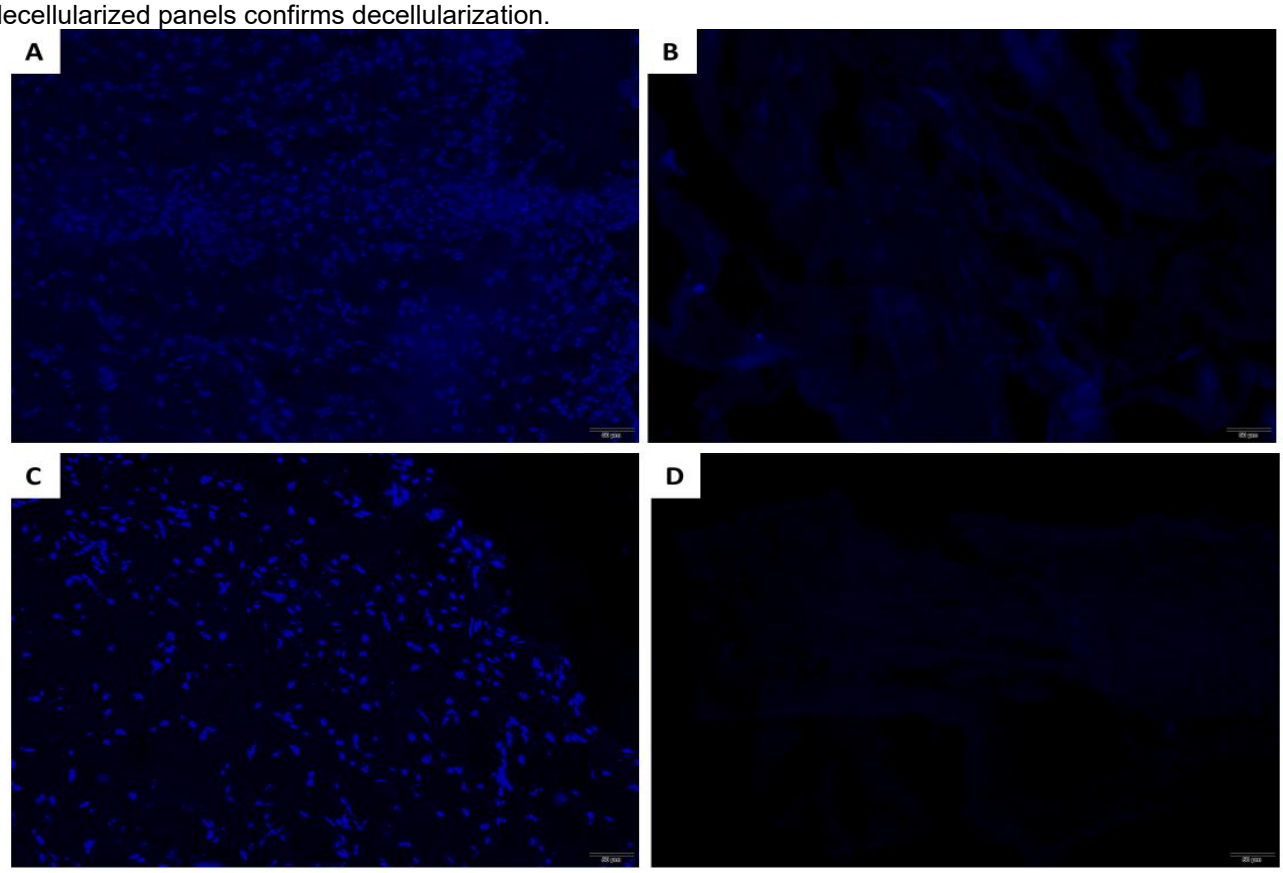
This difference is consistent with the developmental stage of each tooth and may be relevant to the angiogenic potential of the resulting scaffold. In M2, H&E.

Figure 3 - Histological characterization. Open arrows indicate the directly related image. (A) H&E, native M1 - black arrows highlight blood vessels lined by endothelial cells; (B) H&E, native M2 - immature or forming vasculature, not lined by endothelial cells; (C) Masson's trichrome, native M2; (D) H&E, decellularized M1; (E) H&E, decellularized M2; (F) Masson's trichrome, decellularized M2; (G) H&E, decellularized M2, highlighting the preservation of a blood vessel structure; (H) Masson's trichrome, native M1 - arrows highlight blood vessels; (I) Masson's trichrome, decellularized M1. The absence of nuclei in the decellularized panels confirms decellularization. Scale bars 100 µm; 10x magnification (except field G, 20x, 50 µm).



Source: Weiss JB, et al., 2026.

Figure 4 - Fluorescence microscopy (DAPI) of the experimental groups. (A) native M1; (B) decellularized M1; (C) native M2; (D) decellularized M2. Scale bar 50 µm; 20x objective. The absence of nuclei in the decellularized panels confirms decellularization.

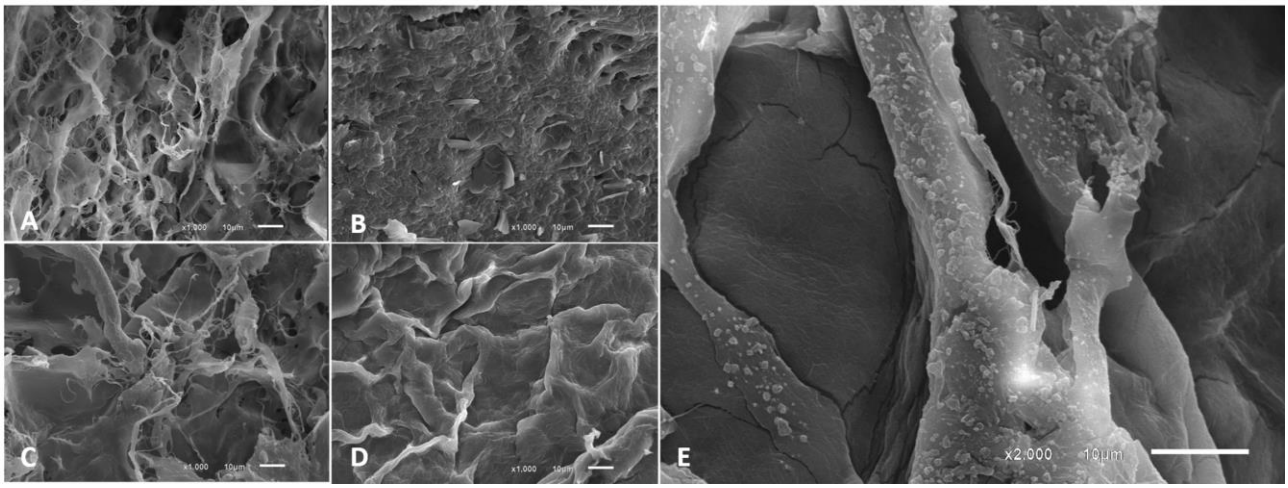


Source: Weiss JB, et al., 2026.

Scanning electron microscopy

Both decellularized groups showed a porous scaffold with efficient cell removal. Native M2 pulps showed crystalline structures suggestive of mineralization intrinsic to tooth formation, and mineralized deposits were observed exclusively in the decellularized M2 group, absent in the other groups (**Figure 5**).

Figure 5 - Scanning electron microscopy. (A) native M2; (B) decellularized M2; (C) native M1; (D) decellularized M1, scale bar 10 µm, 1,000× magnification. (E) Detail of the mineralized deposits remaining in the decellularized M2 group, observed exclusively in this group, scale bar 10 µm, 2,000× magnification.



Source: Weiss JB, et al., 2026.

DISCUSSION

The choice of swine pulp is supported by the availability of the material, a by-product of large-scale slaughter worldwide, and by the anatomical and genetic proximity between pigs and humans (EFTEKHAR M, et al., 2022). The well-established use of porcine tissues in xenotransplantation supports further research on these tissues (YUE Y, et al., 2021).

In contrast to Bakhtiar H, et al. (2021), who worked with bovine pulp, the present study used swine pulp, which may add knowledge for the manufacture of hydrogels and scaffolds. Tomographic examination showed that collecting M2 from the mandible is also possible. However, mandibular bone is naturally much harder than maxillary bone, and upper M2 teeth are embedded in trabecular bone, which is easier to access. M2 pulps were collected from the maxilla only.

This restriction was accounted for arithmetically in **Table 1**, in which yield is expressed as mean weight per pulp and is therefore normalized to the number of teeth actually collected in each group, so that the M1-M2 comparison is not distorted by the different number of teeth obtained per head. Even so, the absence of mandibular M2 teeth remains a limitation, since possible maxillary-mandibular differences in pulp volume and composition at this developmental stage were not assessed.

The absence of a gold standard decellularization protocol is well documented: the systematic review by Elnawam H, et al. (2024) pointed to methodological heterogeneity and high risk of bias across studies. A conservative protocol was chosen, with low-concentration SDS, without Triton X-100 and without nucleases, in order to preserve collagen and laminin. Triton was avoided because of its residual cytotoxicity and its potential to degrade ECM components (GILPIN A e YANG Y, 2017).

Not using nucleases did not compromise DNA removal, which was achieved in both groups. Few studies have evaluated tooth germ pulp; Traphagen SB, et al. (2012) worked with porcine M2 and M3 tooth buds but did not compare developmental stages or fully formed tissues, and their demineralization protocol may have erased mineralization signals in the dental pulp, such as those observed here.

Studies using porcine tooth buds for complete tooth regeneration preserved ECM gradients and demonstrated recellularization and formation of dental tissues in vivo, but did not characterize the decellularized pulp of these tooth germs in detail, which is the focus of the present work (ZHANG W, et al., 2009).

Differences between M1 and M2 in the response to decellularization may relate to vascular architecture. In cat canines, vessels occupy about 14.4% of the coronal pulp area, reaching 42.9% in the pulp core (VONGSAVAN N e MATTHEWS B, 1992). No specific studies on vascularization in pigs were found. One hypothesis is that the greater vascular organization observed in M1 favoured diffusion of the decellularizing solutions into deeper regions of the tissue.

Histological analysis confirmed this contrast in vascular maturity: M1 pulps contained distinct blood vessels lined by endothelial cells, whereas M2 pulps showed immature vasculature still forming and not endothelialized.

This contrast reflects the developmental stage of each tooth. The more mature vascular structures in M1 may represent a relevant feature for future recellularization and angiogenesis strategies; however, this hypothesis depends on confirmation by functional assays, including endothelial immunostaining, recellularization tests and evaluation of the cellular response on the matrix.

The mineralized deposits exclusive to M2 are consistent with active mineralization during tooth formation: the mature pulp ECM is normally non-mineralized, but pulp cells can regulate mineralization under stimulus, with hydroxyapatite deposition associated with type I collagen, closer to the forming crown (LINDE A, 1995).

Although compositional analysis, for example by EDS, was not performed, the morphology observed in the M2 group is consistent with mineralized deposits described previously (HE R, et al., 2022). Scaffold pore size plays a direct role in cell adhesion during recellularization, since each cell type has an ideal size range for efficient adhesion to the scaffold (O'BRIEN FJ, et al., 2005).

Excessively porous scaffolds or those with matrix degradation tend to compromise this adhesion. The porosity of the matrices obtained in this study was not quantified, a step necessary to define the most appropriate recellularization strategy for each pulp type.

The mineralization observed in native M2 can be interpreted in the light of mechanisms already described for pulp tissue. Growth factors and cytokines produced by pulp cells themselves regulate mineralization even outside pathological contexts (COOPER PR, et al., 2014), and mechanical tension in fibronectin fibres is known to directly regulate osteogenesis of mesenchymal stem cells, with more stretched fibres favouring mineralizing differentiation (LI B, et al., 2013).

Calcium availability in the microenvironment has been identified as a determining factor in the differentiation of pulp stem cells (RATHINAM E, et al., 2021). The mineralized deposits observed in M2 may be relevant for applications in mineralized tissues or at the dentin-pulp interface, but their influence on cell adhesion, differentiation and the formation of functional pulp tissue still needs to be investigated.

DNA quantification showed reductions of 92.7% in M1 and 96.8% in M2, close to those reported for effective decellularization, for example around 99.5% in Wolf MT, et al. (2012), although the present values were obtained with a more conservative, nuclease-free protocol.

The convergence of both groups to low residual DNA levels, regardless of the initial differences in cellularity, reinforces the reproducibility and effectiveness of the protocol. Widely cited criteria for decellularization suggest residual DNA below 50 ng/mg dry weight (CRAPO PM, et al., 2011), a threshold reached in both groups.

The relative increase in collagen after decellularization was also reported by Yuan S, et al. (2023). Here, the mean rose from 9.3 to 21.5 µg/mg, following this pattern, although without statistical significance in the post-hoc test, which probably reflects the small sample size and the variability between replicates. The macroscopic appearance, with a change from red to translucent milky white, was consistent with previous reports (TAN Q, et al., 2021).

From an applied standpoint, the choice between the two matrix types depends on the objective of the intended regenerative strategy. The greater yield per tooth in M2, even when restricted to maxillary teeth, favours protocols that demand a large volume of material, such as the production of ECM-derived hydrogels and bioinks, in which yield per collected unit has a direct impact on process viability.

The mature, endothelialized vascular architecture observed in M1, in turn, may be advantageous when the aim is to reproduce a pre-formed vascular bed that guides neoangiogenesis after implantation. This distinction only becomes evident when pulps from different stages are processed under the same protocol, a comparison that had not been described in the literature consulted.

A further practical implication concerns the logistics of obtaining the material. Because M2 molars are intraosseous, their collection requires removal of the bone plate and is therefore more laborious and slower than the collection of erupted M1 molars, even though the yield per tooth is substantially higher. In routine slaughterhouse conditions, where processing time is limited, this trade-off between yield and collection effort should be considered when planning the scale of scaffold production.

The results suggest that the developmental stage of the tooth influences not only the initial cellularity of the pulp but also the organization of the extracellular matrix. M2 showed greater tissue volume, greater cellularity and preservation of collagen content after decellularization.

These differences indicate that matrices obtained from teeth at different developmental stages may present distinct biological properties, an aspect relevant to future applications in regenerative endodontics and tissue engineering.

This study has five main limitations. First, the exact genetic composition and chronological age of the animals were not recorded, which restricts the control of potentially relevant biological variables. Second, M2 pulps were collected from the maxilla only; this was accounted for arithmetically in the calculation of mean weight per pulp (**Table 1**), but possible maxillary-mandibular differences in pulp.

CONCLUSION

The conservative, enzyme-free protocol was effective in decellularizing pulps from fully formed (M1) and developing (M2) swine molars, producing a marked reduction in residual DNA and preserving a porous extracellular matrix scaffold. M2 pulps, collected from the maxilla only, showed greater yield per tooth, higher initial cellularity and mineralized deposits, whereas M1 pulps showed more mature vascularization. These findings indicate that the developmental stage of the tooth should be considered in the selection of matrices for future applications in regenerative endodontics. However, additional studies of porosity, mineral composition, recellularization and implantation are necessary to confirm the functional potential of these scaffolds.

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DECLARATION ON THE USE OF GENERATIVE ARTIFICIAL INTELLIGENCE

During the preparation of this manuscript, the authors used an artificial intelligence tool (Claude, Anthropic) to assist in drafting, reviewing and formatting parts of the text. The authors reviewed and edited all generated content and take full responsibility for the accuracy and integrity of the manuscript.

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