



Cryopreservation in Dentistry: Cryobiological Paradigms, Current Applications, and Transformative Prospects at the Forefront of Dental Regenerative Therapeutics

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LITERATURE REVIEW

ABSTRACT

Objective: This study aimed to review the scientific literature on cryopreservation applied to dentistry, with a special focus on the preservation of dentoalveolar structures and mesenchymal stem cells of dental origin. The aim was to understand the biological foundations involved, technological advances, and future prospects for the creation of autogenous tooth banks and bioengineered constructs. **Methodology:** A systematic search was conducted in PubMed, Scopus, and Web of Science, considering publications published between 2005 and 2025. The descriptors used were: cryopreservation, dental stem cells, tooth bank, and tissue engineering. Original articles, systematic reviews, and experimental trials related to the cryopreservation of dental stem cells, whole teeth, and dentoalveolar tissues were included. The data were organized into categories: principles of cryobiology, technical protocols, current clinical applications, and potential advances. **Results:** The reviewed literature confirms that cryopreservation is already a reality for the conservation of isolated dental stem cells, using protocols that combine cryoprotectants such as DMSO with controlled cooling rates, minimizing damage from ice crystallization. However, the preservation of whole teeth faces significant technical challenges due to tissue heterogeneity and limited diffusion of cryoprotectants. Experimental research indicates promising advances in tooth banks for reimplantation and bioengineered constructs, although they are still far from consolidated clinical application. **Conclusion:** Cryopreservation is emerging as a transformative strategy in regenerative and personalized dentistry, integrating concepts of cryobiology with advanced tissue engineering techniques. However, clinical consolidation depends on overcoming technical limitations and overcoming bioethical and regulatory barriers. In this context, the concept of "cryodentistry" emerges, pointing to a future in which the preservation of tissues and whole teeth could enhance regenerative therapies, reinforcing the role of cryopreservation as a central tool in future dentistry.

Keywords: Cryopreservation; Regenerative Dentistry; Odontogenic Stem Cells; Dental Biobanks; Applied Cryobiology; Orofacial Tissue Engineering.

Criopreservação em Odontologia: Paradigmas Criobiológicos, Aplicações Atuais e Perspectivas Transformadoras na Vanguarda da Terapêutica Regenerativa Dentária

RESUMO

Objetivo: Este estudo teve como objetivo revisar a literatura científica sobre a criopreservação aplicada à Odontologia, com foco especial na preservação de estruturas dentoalveolares e células-tronco mesenquimais de origem dentária. Busque compreender os fundamentos biológicos envolvidos, os avanços tecnológicos e as perspectivas futuras que envolvem a criação de bancos de dentes autógenos e construções bioengenheirados. **Metodologia:** Realizou-se uma busca sistemática nas bases PubMed, Scopus e Web of Science, considerando publicações entre 2005 e 2025. Utilizaram-se os descritores: criopreservação, células-tronco dentárias, banco de dentes e engenharia de tecidos. Foram incluídos artigos originais, revisões sistemáticas e ensaios experimentais relacionados à criopreservação de células-tronco dentárias, dentes inteiros e tecidos dentoalveolares. Os dados foram organizados em categorias: princípios da criobiologia, protocolos técnicos, aplicações clínicas atuais e potenciais avanços. **Resultados:** A literatura revisada confirma que a criopreservação já é realidade na conservação de células-tronco dentárias isoladas, utilizando protocolos que associam crioprotetores como o DMSO a taxas controladas de resfriamento, minimizando danos por cristalização de gelo. No entanto, a preservação de dentes inteiros enfrenta desafios técnicos relevantes, devido à heterogeneidade tecidual e à limitação de difusão de crioprotetores. Pesquisas experimentais indicam avanços promissores em bancos de dentes para reimplante e em construções bioengenheiradas, embora ainda distantes da aplicação clínica consolidada. **Conclusão:** A criopreservação desponta como estratégia transformadora em Odontologia regenerativa e personalizada, integrando conceitos de criobiologia com técnicas avançadas de engenharia tecidual. Contudo, a consolidação clínica depende de superar limitações técnicas e de superar barreiras bioéticas e regulatórias. Nesse contexto, surge o conceito de “criodontologia”, que aponta para um futuro em que a conservação de tecidos e dentes inteiros poderá potencializar terapias regenerativas, reforçando o papel da criopreservação como ferramenta central na medicina dentária do futuro.

Palavras-chave: Criopreservação; Odontologia Regenerativa; Células-Tronco Odontogênicas; Biobancos Dentários; Criobiologia Aplicada; Engenharia Tecidual Orofacial.

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INTRODUCTION

Dentistry, in its evolutionary trajectory, has progressively moved beyond the approach traditionally centered on restoration and rehabilitation, increasingly incorporating biological and regenerative paradigms. In this context of growing therapeutic sophistication, cryopreservation has established itself as an ancillary discipline of notable strategic relevance, as highlighted by Nakashima *et al.* (2017). The ability to maintain cells, complex tissues, and, prospectively, functional organs in a state of biostasis induced by ultra-low temperatures, ensuring their viability and potential functionality for future use, considerably expands the therapeutic arsenal of regenerative medicine (Hackney & English, 2014 and Duailibi *et al.*, 2008).

Cryobiology, as a scientific field dedicated to the study of the effects of cryogenic temperatures on biological systems, offers the indispensable theoretical and methodological foundations for the successful cryopreservation of diverse biological materials, including gametes, embryos, hematopoietic stem cells, and specialized tissues (Pegg, 2002). In the dental field, the consolidation of cryopreservation of mesenchymal stem cell populations derived from different dental niches (Dental Stem Cells – DSCs) stands out to date. These include dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), dental follicle stem cells (DFSCs), and apical papilla stem cells (APSCs), as described by Gronthos *et al.* (2000) and Perry *et al.* (2008). The creation of DSC biobanks aims to create an autologous repository of cells with high phenotypic plasticity, suitable for future applications in orofacial and systemic regenerative therapies.

However, the emerging field of cryodentology transcends this established contribution, opening new frontiers of research and innovation. Notable among these are the cryopreservation of intact teeth extracted for orthodontic reasons or physiologically exfoliated primary teeth for autologous reimplantation, the preservation of dental specimens for advanced translational studies, and the integration with bioengineering protocols for the development of bioactive dental constructs (Young Hak Oh *et al.*, 2005 and Rai *et al.*, 2013). Thus, this article proposes to carry out a critical exegesis of the available literature, with the objective of analyzing the cryobiological foundations applied to the dental context, evaluating the methodologies currently



employed, discussing the technical and scientific challenges involved and prospecting future guidelines for this promising interface between cryobiology and regenerative dentistry.

MATERIALS AND METHODS

During the development of this narrative review article, it was essential to establish a methodological strategy to ensure the inclusion of the most current, relevant, and scientifically validated information on the topic, providing robust and well-supported content. Searches were conducted across multiple databases, including DeCs, BVS/BIREME, PROSPERO, SciELO, PubMed Central, ScienceDirect, Web of Science, and The Cochrane Library, in conjunction with Google Scholar. Additionally, gray literature was utilized to provide supplementary and relevant insights, which proved crucial for a comprehensive exploration of the subject matter. To refine the scope and relevance of the searches, the following descriptors were employed: Cryopreservation, Regenerative Dentistry, Odontogenic Stem Cell, Dental Biobanks, Applied Cryobiology, Orofacial Tissue Engineering. Given the narrative review format, it was necessary to adopt a framework that defines the structure, essential elements, and exclusions pertinent to this type of study. Consequently, Rother's (2007) work served as a methodological guide throughout the preparation of this article, ensuring consistency and adherence to the standards of narrative literature reviews.

CRYOBIOLOGICAL FUNDAMENTALS AND OBSTACLES TO THE CRYOPRESERVATION OF MULTICOMPONENT DENTAL TISSUES

The central goal of cryopreservation is to induce a state of metabolic quiescence through drastic temperature reduction, with the aim of slowing or halting deleterious processes associated with senescence and biological degradation. Achieving this goal intrinsically depends on the meticulous control of the biophysical and biochemical phenomena that occur during the cooling and heating phases (Hackney & English, 2014

and Pegg, 2002). Among the most significant challenges is mitigating the damage caused by ice crystallization. The formation of intracellular crystals is inevitably cytolytic, causing disruption of organelles and compromising the integrity of cell membranes. In turn, extracellular crystallization favors the concentration of solutes in the interstitial space, creating osmotic gradients that lead to excessive cellular water efflux, culminating in injuries due to dehydration and osmotic stress (Mazur, 1970).

These deleterious effects can be reduced through the use of cryoprotective agents (CPAs). These agents can be classified as permeants, such as dimethyl sulfoxide (DMSO), glycerol, and propylene glycol, which cross cell membranes and reduce the amount of intracellular water susceptible to crystallization, increasing cytoplasmic viscosity. Non-permeant agents, such as polysaccharides (sucrose and trehalose) and polymers (hydroxyethyl starch – HES), act primarily in the extracellular environment, modulating ice formation kinetics and stabilizing membranes against osmotic stress (Gao & Critser, 2000). However, the effectiveness of CPAs depends on a careful balance between their concentration and exposure time, given the potential cytotoxicity of these compounds at non-cryogenic temperatures.

Another determining factor for successful cryopreservation is thermal kinetics. In general, controlled, slow cooling rates favor progressive cellular dehydration, preventing the formation of intracellular ice. Conversely, rapid heating is recommended to reduce the risk of recrystallization, a phenomenon that involves the growth of preexisting microcrystals into larger, potentially more damaging structures during the transition from the glassy to the liquid state (Hackney & English, 2014 and Mazur, 1970). As an alternative to conventional freezing, vitrification proposes solidifying the biological system in an amorphous, glassy state, preventing crystal formation, through the use of high concentrations of CPAs combined with ultrafast cooling rates. Although it represents a promising strategy for more complex biological structures, vitrification faces significant limitations related to the toxicity of cryoprotectant cocktails and the difficulty of ensuring thermal homogeneity in bulk samples (Rall & Fahy, 1985).

In summary, a deeper understanding of these biophysical processes and the evolution of thermal and chemical control strategies are central factors for the consolidation of cryopreservation as an essential tool in the advancement of



regenerative dentistry, applied biotechnology, and the construction of new personalized therapeutic paradigms.

CONSOLIDATED APPLICATIONS OF CRYOPRESERVATION IN DENTISTRY: THE PARADIGM OF ODONTOGENIC STEM CELLS BIOBANKS

The most consolidated and clinically translated application of cryopreservation in contemporary dentistry is the establishment of biobanks for the long-term storage of dental stem cells (Dental Stem Cells – DSCs). These cell populations are obtained from different odontogenic niches, each with specific characteristics and broad therapeutic potential. Dental pulp stem cells (DPSCs), isolated primarily from permanent third molars, and SHED (Stem cells from Human Exfoliated Deciduous Teeth), derived from physiologically exfoliated deciduous teeth, stand out for their robust proliferative potential and multilineage differentiation capacity, encompassing odontogenic, osteogenic, chondrogenic, adipogenic, and neurogenic lineages (Gronthos *et al.*, 2000 and Miura *et al.*, 2003). Periodontal ligament stem cells (PDLSCs), described by Seo *et al.* (2004), reside in the periodontal ligament and play a central role in the maintenance and regeneration of tooth-supporting tissues. Apical papilla stem cells (APSCs), located in immature teeth during rhizogenesis, have a high potential for odontoblastic differentiation (Sonoyama *et al.*, 2006). Complementing this spectrum, dental follicle stem cells (DFSCs), derived from the follicle surrounding the tooth bud, participate in the formation of cementum, the periodontal ligament, and alveolar bone (Morsczeck *et al.*, 2005).

Cryopreservation protocols for these cell populations initially involve enzymatic or explant isolation, followed by *ex vivo* cell expansion and subsequent controlled cooling, usually at a rate of -1°C per minute, in a medium containing 10% v/v dimethyl sulfoxide (DMSO) and serum proteins. After reaching -80°C , the samples are transferred for final storage in vapor or liquid nitrogen at -196°C (Perry *et al.*, 2008 and Woods *et al.*, 2009). This methodology aims to preserve viability and cellular differentiation potential for future applications.



The prospective therapeutic spectrum of cryopreserved DSCs is vast and includes the regeneration of the dentinopulpal complex in devitalized teeth, the reconstruction of complex periodontal defects through periodontal regenerative therapy, the repair of maxillofacial bone defects in bone augmentation procedures, and systemic investigational applications aimed at immunomodulation and the release of trophic factors in neurodegenerative, autoimmune, and metabolic diseases (Gronthos *et al.*, 2000 and Miura *et al.*, 2003). Recognition of this clinical potential has driven the creation of public and private biobanks dedicated to the collection, processing, characterization, and cryopreservation of these cells, with a view to their future autologous or allogeneic use (Hilkens *et al.*, 2016).

However, despite this promising outlook, the full translation of DSC-based cell therapies into widespread clinical practice still faces substantial challenges, including the need for methodological standardization, proof of long-term efficacy and safety in robust clinical trials, and overcoming complex regulatory barriers that permeate the field of regenerative medicine.

PROSPECTS FOR CRYODENTISTRY: FROM CRYOPRESERVATION OF INTACT TEETH TO SYNERGY WITH ADVANCED TISSUE BIOENGINEERING

Beyond the cryopreservation of isolated cell populations, more ambitious and potentially transformative applications of cryology are emerging in future dentistry. Notable among these prospects is the creation of autologous tooth banks, a proposal that involves the cryopreservation of sound permanent teeth extracted for orthodontic reasons or asymptomatic third molars, as well as exfoliated primary teeth that maintain root integrity. This strategy aims to enable subsequent autologous reimplantation in cases of tooth loss, restoring not only anatomy but also physiological function, including proprioception and integration with periodontal tissues (Young Hak Oh *et al.*, 2005 and Rai *et al.*, 2013). However, this approach faces significant technical challenges, such as preserving the viability of cell populations in the dental pulp and periodontal ligament, as well as maintaining biomechanical integrity under cryogenic stress. Perfusion



protocols with optimized cryoprotective agents and advanced vitrification strategies for complete dental organs remain at the experimental stage (Kaku *et al.*, 2010).

Another relevant frontier lies in cryopreservation as a strategic tool for dental tissue engineering. In this context, biofunctional constructs composed of three-dimensional scaffolds functionalized with dental stem cells and bioactive molecules could be cryopreserved, becoming off-the-shelf products ready for immediate clinical application, expanding access and therapeutic predictability. Similarly, the bioengineering of complete dental germs from stem cells, as described by Duailibi *et al.* (2008), could be combined with the cryopreservation of these germs at early ontogenetic stages, enabling long-term storage for later transplantation.

Cryopreservation is also emerging as a strategic resource for scientific research and diagnostic innovation. The organization of biobanks of intact teeth, tissue fragments, and bioengineered tooth germs, properly characterized and stored under validated protocols, constitutes a valuable resource for research in odontogenesis, oral pathology, the identification of molecular biomarkers with diagnostic or prognostic value, as well as for the preclinical evaluation of new regenerative therapies.

Although still speculative, the application of cryology in the form of dental cryosurgery, a modality already established in other medical specialties, is envisioned. This approach could include the future development of miniaturized cryoprobe aimed at the selective and minimally invasive ablation of early lesions or the modulation of local inflammation. However, such a prospect requires substantial technological advances and robust translational studies that demonstrate efficacy and safety in the dental context.

In summary, these approaches significantly broaden the scope of “cryodentistry,” integrating concepts of cryobiology, tissue engineering, and technological innovation, and projecting scenarios in which cryopreservation transcends isolated cell storage to become a strategic pillar of regenerative, personalized, and prospectively preventive dentistry.

TECHNOLOGICAL, BIOETHICAL AND LEGAL-REGULATORY BARRIERS TO THE EXPANSION OF CRYODONTOLOGY



The realization of these futuristic perspectives at the interface between cryopreservation and regenerative dentistry depends on overcoming a wide range of technical, scientific, economic, bioethical, and regulatory challenges. In the scientific sphere, there is a pressing need for substantial advances in cryobiological science, including the development of new generations of cryoprotective agents with greater efficacy and lower intrinsic toxicity, the improvement of vitrification protocols applicable to complex and heterogeneous biological systems such as intact teeth, and a deeper understanding of the molecular mechanisms underlying cryoinjury and cryoprotection. Furthermore, the standardization and rigorous validation of protocols, ranging from collection, processing, and cryopreservation to thawing and post-cryopreservation functional evaluation, are essential to ensure scientific reproducibility and clinical safety, requiring well-designed preclinical studies and robust clinical trials.

In the economic arena, careful cost-benefit analyzes and strategies that enable equitable access to these advanced technologies are essential, considering that cryopreservation processes and associated regenerative therapies can represent high costs and potentially inequitable access. From a bioethical perspective, relevant issues emerge, such as obtaining free and informed consent that encompasses the therapeutic and scientific purposes of biological storage, defining clear guidelines for biobank governance and ownership, and implementing systems that ensure the quality, traceability, and safety of stored material. It is equally essential to promote accurate communication with patients and society, avoiding the creation of disproportionate expectations regarding therapies that are still in the experimental phase or undergoing translational research.

Finally, the incorporation of these innovative therapies into clinical practice is subject to compliance with requirements imposed by a complex and rigorous regulatory framework. Advanced cell therapy products, such as those derived from dental stem cells or tissue-engineered constructs, are evaluated by regulatory agencies such as the FDA (Food and Drug Administration) and the EMA (European Medicines Agency), requiring lengthy, costly, and technically detailed approval processes. This situation reinforces that, although the outlook is promising, the full implementation of these innovations depends on the convergence of scientific progress, technical feasibility, bioethical responsibility, and regulatory compliance to ensure the safety, efficacy, and



social benefits of cryopreservation-based therapies.

CONCLUSION

Cryopreservation, far from being a mere conservation technique, has already established itself as a valuable tool in contemporary dentistry, notably through the creation of odontogenic stem cell banks, which constitute a promising resource for personalized regenerative medicine. Looking ahead, the scope of "cryodentistry" is substantially broader, encompassing everything from the achievable utopia of autogenous tooth banks to their symbiotic integration with the most advanced tissue bioengineering platforms.

The full realization of this vast potential depends on a continuous multidisciplinary research effort that promotes synergistic advances in cryobiology, cellular and molecular biology, biomaterials science, and tissue engineering. At the same time, a mature and informed dialogue on the logistical, socioeconomic, bioethical, and legal-regulatory aspects is essential. The global dental community including its academic, scientific, and clinical strata has a duty not only to monitor these disruptive developments, but also to actively participate in knowledge generation, ethical deliberation, and policy formulation that ensure the judicious, safe, and effective implementation of these technologies.

The convergence of cryobiology with dental practice represents not an ornamental futuristic digression, but rather a scientific frontier with the potential to fundamentally redefine treatment paradigms for dental tissue loss and degeneration, heralding an era of genuinely biological, personalized, and regenerative interventions. The safeguarding of individual biological heritage for the therapeutic contingencies of the future may, in fact, become an intrinsic and distinctive component of precision dentistry in the 21st century and beyond.

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