

牙本质基质材料在牙和骨组织再生中的应用

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收稿日期: 2025年1月26日; 录用日期: 2025年2月19日; 发布日期: 2025年2月27日

摘 要

损伤组织的修复与再生一直是生物学和医学领域的重要研究课题。牙齿和骨组织缺损是临床中常见的疾病问题。与软组织不同, 牙齿和骨等硬组织的再生修复具有其独特的特点。支架在组织工程中起着至关重要的作用, 为组织修复与再生提供必要的微环境。牙本质基质是一种无细胞的天然生物活性支架, 具有优异的生物相容性, 已广泛应用于硬组织的再生修复。本文综述了牙本质基质的生物学特性、材料种类及其在组织再生中的应用, 为未来牙本质基质材料的基础研究与临床应用提供参考。

关键词

牙本质基质, 牙本质再生, 骨再生, 牙周再生

Applications of Dentin Matrix Materials in Tooth and Bone Tissue Regeneration

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Received: Jan. 26th, 2025; accepted: Feb. 19th, 2025; published: Feb. 27th, 2025

Abstract

The repair and regeneration of damaged tissues have long been a key focus of research in biology and medicine. Tooth and bone tissue defects are common clinical issues. Unlike soft tissues, the regeneration and repair of hard tissues, such as teeth and bones, involve distinct processes. Scaffolds play a crucial role in tissue engineering by providing the necessary microenvironment for tissue repair and regeneration. The dentin matrix is a cell-free, natural bioactive scaffold with excellent

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biocompatibility, widely used in the regeneration and repair of hard tissues. This article reviews the biological properties of the dentin matrix, its material types, and applications in tissue regeneration, offering a reference for future basic research and clinical applications of dentin matrix materials.

Keywords

Dentin Matrix, Dentin Regeneration, Bone Regeneration, Periodontal Regeneration

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1. 引言

牙齿和骨是人体重要的器官,属于高度矿化的组织,且是运动系统的关键组成部分。骨的主要功能包括支持、运动、保护身体,参与造血以及储存矿物质。牙齿不仅具有咀嚼、辅助发音和言语的功能,还能保持面部形态的协调与美观。当牙槽骨因感染、创伤、病理或牙周疾病发生缺失时,骨移植是一种有效的再生方法[1],自体骨被认为是骨缺损再生的“金标准”,但自体骨的来源有限。牙本质基质材料已被制备,研究表明其可作为骨再生的替代材料。牙本质不含细胞,且其来源广泛。例如,因正畸需求或阻生而拔除的完整牙齿通常作为医疗废物处理,但实际上它们提供了有价值的来源,且作为骨移植供体时,其相关发病率较低[2]-[4]。牙本质作为牙齿的主要成分,其理化性质与骨相似。牙本质和骨的成分相似,均由约70%的磷酸钙、18%的胶原蛋白、2%的非胶原蛋白及10%的水组成[5]。在组织学结构上,牙本质主要由生物磷灰石晶体沉积在I型胶原纤维上,形成梯度结构,类似于骨的结构[5][6]。牙本质作为一种天然的无细胞基质材料,除了胶原蛋白外,还含有非胶原蛋白,如生长因子、蛋白聚糖和小整合素结合配体N-连接糖蛋白(small integrin-binding ligand N-linked glycoproteins, SIBLINGs),这些成分已被证明有利于骨再生[7]。因此,经过适当处理的牙本质可以作为生物活性的骨移植材料。此外,牙周病、龋齿、机械创伤及牙髓并发症等引起的牙齿缺损是常见病,严重影响人们的生活质量。近年来,牙本质因其具有用于牙再生[8][9]和骨组织重建[10][11]的生物活性细胞外基质而备受关注。脱细胞基质(acellular matrix, ACM)因其无免疫原性和优越的机械性能,已广泛应用于组织工程中的支架材料[8]。作为生物支架材料,牙本质基质(dentin matrix, DM)能为种子细胞提供三维生长空间,并模拟细胞所需的细胞外基质,促进细胞黏附与增殖,从而影响组织形成[12]。本文简要介绍了牙本质基质材料的特性、主要种类及其在组织再生中的应用,为未来相关研究提供参考。

2. 牙本质基质(DM)的特性

牙本质是一种矿化组织,由牙本质小管和细胞间质构成,是构成牙体的主要硬组织,主要功能是保护牙髓并支持表面的釉质。人牙本质大约由70%的羟基磷灰石、20%的基质蛋白和10%的水组成。牙本质含有一类独特的磷蛋白家族,包括牙本质唾液磷蛋白(dentin sialophosphoprotein, DSPP)、牙本质基质蛋白1(dentin matrix protein 1, DMP1)、骨唾液蛋白(bone sialoprotein, BSP)、基质细胞外磷酸化糖蛋白(matrix extracellular phosphorylated glycoprotein, MEPE)和骨桥蛋白(osteopontin, OPN)[13][14]据报道,DSPP无效突变小鼠的牙本质矿化受到破坏,因此,DSPP基因产物在牙本质矿化形成过程中发挥着特定且关键的作用[15]。牙本质还含有胎球蛋白-A,这是一种在肝脏和矿化组织(如牙本质)中产生的血清蛋白,能够抑制

异位钙化, 同时不影响牙本质或骨的矿化过程[16]。

牙本质基质(DM)由胶原蛋白(约86%的I型以及III、V和VI型)和非胶原蛋白(non-collagenous proteins, NCPs)组成[17], 含有丰富的生物活性生长因子和细胞外基质蛋白, 参与牙髓祖细胞的募集、增殖和分化[18]。薄的胶原原纤维构成前牙本质, 与骨的类骨质基质相似。NCPs可能在前牙本质转化为牙本质的过程中发挥作用, 有研究表明, 一些NCPs能促进羟基磷灰石(hydroxyapatite, HAP)晶体的生长, 其效果受浓度和构象形式的影响。此外, 突变研究也表明, NCP在牙本质和/或骨的矿化过程中是不可或缺的[13]。DM中含有丰富的类似骨基质的生长因子[19], 如牙本质基质蛋白(DMP)-1、DMP-4、牙本质唾液蛋白(dentin sialoprotein, DSP)、牙本质磷酸蛋白(dentin phosphoproteins, DPP)等[20]。这些生长因子能够与羟基磷灰石结合, 并能刺激基质蛋白或其他重要分子的产生, 调节再矿化过程[21], 例如, Gericke等人发现, 骨形态发生蛋白(bone morphogenetic protein, BMP)-1与羟基磷灰石矿化相关, 而不同形式的DMP-1共同调控矿化过程[22]。磷酸蛋白(Phosphophoryn, PP)和DSP是牙本质中的两种主要非胶原性细胞外基质, 均来源于DSPP基因。PP在前牙本质向牙本质转化过程中起着重要作用, 是矿化牙齿形成所必需的[23]。DSP既可以作为结构蛋白, 也参与调节羟基磷灰石的形成。免疫定位实验表明, DSP在积极参与牙本质矿化的成牙本质细胞中表达[23][24]。因此, 提出从牙本质中释放这些分子, 作为生物活性分子的来源, 具有修复或恢复牙齿结构的显著潜力[21][25]。作为生物活性支架, 细胞外基质的活性保存仍然是一个巨大的挑战。研究表明, 冷冻处理达6个月的牙本质基质仍保持良好的生物活性, 在生物牙根再生中, 能够像新鲜牙本质基质一样再生牙髓-牙本质组织[26]。这表明牙本质基质是一种极为稳定的脱细胞基质。

3. 牙本质基质(DM)材料的种类

3.1. 脱矿牙本质基质(Demineralized Dentin Matrix, DDM)

脱矿牙本质基质(DDM)是通过机械去除牙釉质、牙骨质和牙髓后, 采用不同的脱矿方法制备而成。例如, 采用不同浓度梯度的乙二胺四乙酸(EDTA)处理不同时间[27]-[29], 使用0.6N HCl在不同时间段暴露[30]-[33], 或用2% HNO₃分别在10分钟[32]和20分钟[34]的时间内脱矿。目前, DDM有粉末和块状两种形式。粉末状DDM的矿物质含量约为5%~10%, 其通过将牙本质粉碎成直径约300~800 μm的颗粒制成; 而块状DDM的矿物质含量约为10%~30%, 保留了牙本质小管等基本结构[6]。不同文献中报道的脱矿剂和脱矿时间各不相同, 从而影响脱矿的程度和效率。除脱矿剂外, 颗粒的大小也会影响脱矿效果。研究发现, 将部分脱矿牙本质基质(partially demineralized dentin matrix, PDDM)植入大鼠颅盖骨缺损后, 完全脱矿的牙本质基质(completely demineralized dentin matrix, CDDM)大部分被再吸收, 而未矿化牙本质(undemineralized dentin, UDD)则被保留。CDDM和UDD均诱导较少的骨形成, 而PDDM则诱导了更多的新骨形成[35]。在几乎所有报告中, DDM和PDDM这两个术语是可互换的, 因为DDM通常是部分脱矿的[36]。脱矿作用可以打开牙本质小管, 植入骨缺损处时, 牙本质小管充当释放生物活性蛋白的通道。UDD中生长因子的暴露有限, 尽管CDDM也具有开放的小管, 但在脱矿过程中, 生物活性蛋白可能已经从牙本质基质中释放, 因此在植入前已丧失。而PDDM相比CDDM可能含有更多的生长因子。脱矿时间的不同也会影响DDM块(DDB)的骨再生效果。研究表明, 采用不同脱矿时间(10分钟, DDB 10; 60分钟, DDB 60)处理的DDM分别移植到大鼠颅盖缺损中。结果显示, DDB 60组在8周时产生的新骨面积显著大于DDB 10组, 但吸收率更高, 导致总的增强面积较小[37]。因此, 脱矿牙本质基质(DDM)作为一种具有优异生物活性的材料, 具有促进骨和牙组织再生的潜力[6][38]。

3.2. 经处理的牙本质基质(Treated Dentin Matrix, TDM)

经处理的牙本质基质(TDM) [8]是一种特殊类型的脱细胞外基质(dECM), 来源于牙本质基质(DM)。

其通常通过以下基本步骤[39]制备: 拔牙、去除牙骨质和牙髓、切片或粉碎成颗粒、脱矿, 并根据需要进行特殊处理[40], 随后灭菌和保存。通常采用梯度 EDTA (17%, 5%) 对牙本质基质进行脱矿, 以暴露牙本质小管[8]。不同文献中 EDTA 的浓度和脱矿时间有所不同[3] [8] [41] [42]。TDM 保留了天然牙本质小管的基本结构, 能够支持干细胞粘附, 并为营养和代谢废物的交换提供必要的空间[39], 此外, TDM 还含有生长因子, 可有效诱导干细胞增殖、粘附和分化, 从而促进牙髓、牙本质、牙骨质、牙周韧带和牙槽骨的再生[9] [43]。此外, TDM 能够利用其暴露的牙本质小管独特的结构, 充当药物分子[44]-[46]和生物活性剂[18] [29] [47]的载体, 并控制其释放。近年来, 有研究将水凝胶(如海藻酸钠)与 TDM 复合, 形成可注射水凝胶用于临床应用。例如, Ahmed A. Holiel 等人将制备的可注射处理牙本质基质水凝胶(TDMH)与商业盖髓材料 Biodentine 和 MTA 进行比较, 以评估其在外伤性暴露恒后牙盖髓手术中的成功率。经过 2 年的随机对照临床试验, 并通过锥形束 CT 成像, 发现 TDMH 相比 Biodentine 和 MTA 具有更大的诱导牙本质桥形成潜力[48]。因此, TDM 是一种有前途的生物材料, 具有广泛的组织再生应用潜力。

3.3. 冻存牙本质基质(Cryopreserved Dentin Matrix, CDM)

冻存牙本质基质(CDM)是将 DDM/TDM [26] [49]置于液氮中保存的生物材料。众所周知, 生物材料可以在低温下长期保存[26] [50], 通过将其冷却至远低于水的玻璃化转变温度(-135°C) [49], 这一过程基本“暂停”了生物活性和分子运动, 从而实现了保存生物构建体并为未来使用储存的能力。冷冻保存技术已在细胞、组织、器官及其他生物构建体的长期保存中取得成功[51]-[53]。此外, 研究表明, 冷冻保存可在一定程度上降低组织的免疫原性[54] [55]。目前, 常用的生物样品保存温度包括室温、 4°C 、 20°C 、 80°C 和 196°C 。通常, 蛋白质、RNA 和细胞储存在 -80°C 或 -196°C , 因为低温损伤主要发生在 0°C 至 60°C 之间[49]。Liang Jiao 等人研究了将 TDM 分别在液氮中保存 3 个月(CDM-3)和 6 个月(CDM-6)后的力学性能、细胞增殖和成牙能力, 结果表明 CDM 的机械性能优于 TDM。与 TDM 相比, CDM 具有更大的孔径, 能释放更多与牙本质形成相关的蛋白。将 CDM 提取液加入细胞培养中, 前 5 天的细胞增殖与 TDM 相似。为进一步评估 CDM 的诱导能力, 研究者将人牙囊细胞包封在 CDM 内, 并将支架植入小鼠模型中 8 周, 结果发现, CDM 提取液能促进人牙囊细胞表达骨涎蛋白、胶原-1 和碱性磷酸酶, 表明 CDM 在牙胚发育中作为诱导性微环境发挥了重要作用。最重要的是, 体内实验表明, CDM 可诱导牙囊细胞再生新型牙本质牙髓样组织, 包括牙本质小管、前牙本质、胶原纤维、神经和血管, 且这些组织对牙本质唾液磷蛋白、牙本质基质蛋白-1、微管蛋白和胶原-1 呈阳性[26]。因此, CDM 是一种理想的生物支架材料, 适用于人牙本质牙髓样组织的再生。这些发现表明, TDM 可作为组织工程支架进行保存, 并且易于应用于患者治疗。此外, TDM 的成功冻存为其他组织工程生物活性支架材料的保存提供了新的思路。

3.4. 消化牙本质(基质)提取物(Digested Dentin Matrix Extract, DDME)

消化牙本质(基质)提取物(DDME)是通过酸性胃蛋白酶溶液处理新鲜人牙本质基质(DM)粉末制得的一种生物材料, 呈白色粉末状颗粒, 平均尺寸约为 $8\text{ }\mu\text{m}$, 与先前报道的牙本质基质粉末相比, DDME 具有更小的直径。电子显微镜下, DDME 与未处理的牙本质粉(GDM)相比, 表面暴露大量多肽结构, 呈现不规则的凹凸形态, 且未见牙本质的基本结构; 而 GDM 则保留了牙本质的基本结构。X 射线光电子能谱(XPS)分析显示, DDME 与牙本质粉末中均可观察到碳、氮、氧、钙和磷的类似峰, 且氮信号(N1s)的强度明显增强, 表明材料表面暴露了更多的肽类物质, 从而增加了外表面面积, 有助于细胞黏附到 DDME 表面。在 DDME 与人牙髓细胞(human dental pulp cells, hDPCs)共培养中, 发现 DDME 具有良好的生物相容性, 并能够诱导细胞成牙分化。在裸鼠皮下异位移植实验中, DDME 可促进 AQP4 和 CD31 的表达升高, 表明其具有促进成牙诱导的能力, 并且在体内仍能有效诱导成牙过程, 可能被机体组织吸收并融合

形成类牙本质结构。在大鼠磨牙原位诱导牙本质形成实验中, DDME 能够与牙髓组织融合, 形成新的类牙本质结构, 表明 DDME 具有良好的成牙诱导能力, 具有作为新型盖髓材料的潜力[56]。

小结: 自体、异体和异种骨材料具有较高的生物活性和骨诱导性, 但也存在来源、免疫原性等潜在风险。例如, 拔牙是制备牙本质基质材料的最常见来源, 常见的动物来源包括牛[40]、犬[43]、猴[43]、猪[46]等。然而, 这些材料可能引发免疫反应。以往的研究表明, TDM 可在含青霉素和链霉素的 PBS 中浸泡 72 小时, 然后在无菌去离子水中清洗, 以达到消毒目的[57], 但仍存在免疫应答和感染的风险。然而, 研究也表明, 去矿化[58]和高温灭菌[59]可以显著降低同种异体牙本质基质的抗原性和免疫原性。目前尚未有研究比较不同牙本质材料的免疫原性是否会对成牙/成骨分化产生影响。

4. 牙本质基质材料的应用

4.1. 牙周组织再生

牙周组织再生是指牙周治疗后, 新的无细胞牙骨在牙质表面或原始牙骨表面沉积, 功能性胶原纤维束在牙骨和牙槽骨之间形成并附着在病变部位, 从而实现牙周组织的再生及功能恢复。牙周组织再生需要牙齿的骨样组织和整齐排列的纤维组织构成的三明治结构。同种来源的牙周膜干细胞与 TDM 的结合是可行的。将牙周膜干细胞膜片与 TDM 复合后, 植入牙齿的单壁牙周骨缺损部位, 能够促进新骨和牙周膜样组织的形成[28]。从人类脱落的乳牙中提取的干细胞(stem cells from human exfoliated deciduous teeth, SHEDs)可作为种子细胞的替代品, 用于牙周组织的再生。研究人员将 SHEDs 衍生的细胞膜与 TDM 结合, 植入大鼠颌骨缺损区, 观察到新生牙周纤维、血管和牙槽骨的形成, 成功再生了牙周组织[60]。然而, 结合功能优良的生物支架与生长因子, 不需要体外细胞培养即可实现原位组织再生。富含血小板的纤维蛋白(PRF)和 TDM 都含有大量生长因子。将 TDM 包覆于 PRF 膜中并植入拔牙窝, 3 个月后可观察到牙骨和牙周膜样组织的形成[61]。

4.2. 牙本质再生

牙本质的脱矿作用暴露了牙本质基质中的有机成分, 尤其是胶原蛋白, 并增强了组织的传导性, 从而促进骨生成或牙本质生成[27]。Sirui Liu 等人实验表明, 不同浓度的 TDM 均能促进 hDPSCs 向牙本质细胞分化, 并降低 GSK 3 β 的表达; 同时, TDM 培养的大鼠下颌骨中可见新生牙本质, 免疫组化染色显示 GSK 3 β 在新生牙本质中的表达较低, 提示 TDM 通过直接靶向 GSK 3 β , 激活经典的 Wnt/ β -catenin 信号通路, 促进 hDPSCs 的牙源性分化[59]。这一现象可能与组蛋白甲基化过程相关。Li 等人发现, zeste 增强子同源物 2 (EZH2)在体外负调节 hDPSCs 的分化, 并抑制移植的 β -磷酸三钙/hDPSCs 复合物中矿化结节的形成; EZH2 缺失激活 Wnt/ β -catenin 信号通路, 增强 hDPSC 中 β -catenin 的积累, 这一作用归因于 EZH2 调节 β -连环蛋白启动子上 H3 K27 me3 的水平[62]。Yang 等人发现, 组蛋白去甲基化酶 JHDM 1D (又称 KDM 7A)敲除后, hDPSCs 矿化结节的形成增加, β -catenin 表达上调, 这一过程是通过下调 DKK1 实现的。抑制 JHDM 1D 的表达可以激活 Wnt/ β -catenin 信号通路, 从而调控 hDPSCs 的牙本质形成[63]。此外, Chen J 等人发现, 同种异体 TDM 支架在高压灭菌后仍具有生物相容性, 能支持干细胞分化为牙本质样组织且无免疫原性, 同时观察到牙釉质再生[57]。Jinlong Chen 等人实验发现, TDM 糊剂在中性 pH 条件下具有较好的生物相容性, 显著促进牙髓干细胞的增殖, 并增加碱性磷酸酶、骨唾液蛋白、牙本质唾液蛋白等基因和蛋白的表达; TDM 糊剂在体内盖髓后, 能够形成连续的修复性牙本质桥, 实现牙本质再生和活髓保存的双重作用[64]。

4.3. 骨组织再生

基于处理过的牙本质基质的生物材料在骨组织再生中的应用, 主要集中于拔牙后的牙槽嵴保存和牙

槽窝保存[64]-[66]。例如, Moraes 等人发现脱矿的人牙本质基质(DDM)有助于牙槽嵴的保存, 显微断层扫描和组织学评估显示, DDM 有助于新骨形成并缓慢重吸收[57]。Li 等人发现自体脱矿牙本质基质在骨增强中表现出成骨效果, 类似于 Bio-Oss®对口腔骨缺损的效果[64]。Murata 等人发现, 部分 DDM/牙骨质基质有助于牙槽窝的保存, 表现为移植区域中的骨样射线不透性和与牙本质/牙骨质区域直接连接的新形成骨的出现[66]。Um 等人发现, 自体 DDM 负载重组人骨形态发生蛋白 2 (BMP-2)有助于牙槽窝的保存, 并增强骨形成的有效性[32]。Reis-Filho 等人发现, 人脱矿质牙本质基质具有成骨诱导潜力, 能促进牙槽内骨组织和血管组织的形成[65]。

5. 结论

综上所述, 牙本质基质包含多种蛋白和活性生长因子, 如脱矿牙本质基质(DDM)、经处理牙本质基质(TDM)、冻存牙本质基质(CDM)和消化牙本质提取物(DDME)。这些材料不仅具有成牙本质诱导活性, 能促进干细胞向牙本质分化, 还具有成骨诱导活性, 有助于牙槽嵴和牙槽骨的保存。此外, 处理过的牙本质基质在牙周再生工程中也发挥着重要作用。尽管已有大量研究探讨其应用和诱导能力, 但只有少数研究关注其潜在机制[27], 这可能限制了对牙本质基质(DM)生物诱导能力的深入理解。目前的研究中, 缺乏对不同成品商业盖髓材料与牙本质基质材料在成牙/成骨、免疫原性和力学性能等方面的比较, 这使得临床应用中的材料选择缺乏指导依据。此外, 现有研究大多采用无病理条件的完整牙齿制备牙本质基质用于组织再生。然而, 牙科疾病导致的拔牙资源具有巨大潜力, 这些患牙值得作为实验对象, 研究其在相同制造程序下(对比是否去除病变)后的物理性质和生物学能力, 从而探索和利用这些被视为医疗废物但具回收潜力的资源。异种牙齿来源同样值得考虑, 例如猪 TDM 已被证实具有与人 TDM 相似的矿物质组成和生物活性分子[42]。最后, 应用受控酶解法制备牙本质基质提取物是一种新兴方法, 具有巨大的研究潜力, 特别是在基质的潜力和诱导组织再生能力方面。

总之, 牙本质基质不仅在牙本质、牙周和骨组织修复中发挥重要作用, 还具有促进其他组织再生的潜力。目前, 其具体作用机制尚不明确, 同时牙本质基质材料仍存在一定缺陷。未来可以通过改性或与其他材料的联合应用, 提升其临床应用价值。

声 明

该病例报道已获得病人的知情同意。

参考文献

- [1] Sultan, N. and Jayash, S.N. (2023) Evaluation of Osteogenic Potential of Demineralized Dentin Matrix Hydrogel for Bone Formation. *BMC Oral Health*, **23**, Article No. 247. <https://doi.org/10.1186/s12903-023-02928-w>
- [2] Kim, Y., Kim, S., Oh, J., Jin, S., Son, J., Kim, S., *et al.* (2011) Analysis of the Inorganic Component of Autogenous Tooth Bone Graft Material. *Journal of Nanoscience and Nanotechnology*, **11**, 7442-7445. <https://doi.org/10.1166/jnn.2011.4857>
- [3] Avery, S., Sadaghiani, L., Sloan, A. and Waddington, R. (2017) Analysing the Bioactive Makeup of Demineralised Dentine Matrix on Bone Marrow Mesenchymal Stem Cells for Enhanced Bone Repair. *European Cells and Materials*, **34**, 1-14. <https://doi.org/10.22203/ecm.v034a01>
- [4] He, Y., Tian, M., Li, X., Hou, J., Chen, S., Yang, G., *et al.* (2021) A Hierarchical-Structured Mineralized Nanofiber Scaffold with Osteoimmunomodulatory and Osteoinductive Functions for Enhanced Alveolar Bone Regeneration. *Advanced Healthcare Materials*, **11**, Article ID: 2102236. <https://doi.org/10.1002/adhm.202102236>
- [5] Wu, X., Peng, W., Liu, G., Wang, S., Duan, B., Yu, J., *et al.* (2023) Extrafibrillarly Demineralized Dentin Matrix for Bone Regeneration. *Advanced Healthcare Materials*, **12**, Article ID: 2202611. <https://doi.org/10.1002/adhm.202202611>
- [6] Um, I., Kim, Y. and Mitsugi, M. (2017) Demineralized Dentin Matrix Scaffolds for Alveolar Bone Engineering. *The Journal of Indian Prosthodontic Society*, **17**, 120-127. https://doi.org/10.4103/jips.jips_62_17

- [7] Bertassoni, L.E. (2017) Dentin on the Nanoscale: Hierarchical Organization, Mechanical Behavior and Bioinspired Engineering. *Dental Materials*, **33**, 637-649. <https://doi.org/10.1016/j.dental.2017.03.008>
- [8] Guo, W., He, Y., Zhang, X., Lu, W., Wang, C., Yu, H., *et al.* (2009) The Use of Dentin Matrix Scaffold and Dental Follicle Cells for Dentin Regeneration. *Biomaterials*, **30**, 6708-6723. <https://doi.org/10.1016/j.biomaterials.2009.08.034>
- [9] Yang, B., Chen, G., Li, J., Zou, Q., Xie, D., Chen, Y., *et al.* (2012) Tooth Root Regeneration Using Dental Follicle Cell Sheets in Combination with a Dentin Matrix-Based Scaffold. *Biomaterials*, **33**, 2449-2461. <https://doi.org/10.1016/j.biomaterials.2011.11.074>
- [10] Andersson, L., Ramzi, A. and Joseph, B. (2009) Studies on Dentin Grafts to Bone Defects in Rabbit Tibia and Mandible; Development of an Experimental Model. *Dental Traumatology*, **25**, 78-83. <https://doi.org/10.1111/j.1600-9657.2008.00703.x>
- [11] Mordenfeld, A., Hallman, M. and Lindskog, S. (2011) Tissue Reactions to Subperiosteal Onlays of Demineralized Xenogenous Dentin Blocks in Rats. *Dental Traumatology*, **27**, 446-451. <https://doi.org/10.1111/j.1600-9657.2011.01026.x>
- [12] Zhang, J., Hu, Z.Q., Turner, N.J., Teng, S.F., Cheng, W.Y., Zhou, H.Y., *et al.* (2016) Perfusion-Decellularized Skeletal Muscle as a Three-Dimensional Scaffold with a Vascular Network Template. *Biomaterials*, **89**, 114-126. <https://doi.org/10.1016/j.biomaterials.2016.02.040>
- [13] Chaussain, C., Boukpepsi, T., Khaddam, M., Tjaderhane, L., George, A. and Menashi, S. (2013) Dentin Matrix Degradation by Host Matrix Metalloproteinases: Inhibition and Clinical Perspectives toward Regeneration. *Frontiers in Physiology*, **4**, Article 308. <https://doi.org/10.3389/fphys.2013.00308>
- [14] Opsahl Vital, S., Gaucher, C., Bardet, C., Rowe, P.S., George, A., Linglart, A., *et al.* (2012) Tooth Dentin Defects Reflect Genetic Disorders Affecting Bone Mineralization. *Bone*, **50**, 989-997. <https://doi.org/10.1016/j.bone.2012.01.010>
- [15] Hayano, S., Kurosaka, H., Yanagita, T., Kalus, I., Milz, F., Ishihara, Y., *et al.* (2012) Roles of Heparan Sulfate Sulfation in Dentinogenesis. *Journal of Biological Chemistry*, **287**, 12217-12229. <https://doi.org/10.1074/jbc.m111.332924>
- [16] Orsini, G., Ruggeri, A., Mazzoni, A., Nato, F., Manzoli, L., Putignano, A., *et al.* (2009) A Review of the Nature, Role, and Function of Dentin Non-Collagenous Proteins. Part 1: Proteoglycans and Glycoproteins. *Endodontic Topics*, **21**, 1-18. <https://doi.org/10.1111/j.1601-1546.2012.00270.x>
- [17] Guirado, E. and George, A. (2021) Dentine Matrix Metalloproteinases as Potential Mediators of Dentine Regeneration. *European Cells and Materials*, **42**, 392-400. <https://doi.org/10.22203/ecm.v042a24>
- [18] Melling, G.E., Colombo, J.S., Avery, S.J., Ayre, W.N., Evans, S.L., Waddington, R.J., *et al.* (2018) Liposomal Delivery of Demineralized Dentin Matrix for Dental Tissue Regeneration. *Tissue Engineering Part A*, **24**, 1057-1065. <https://doi.org/10.1089/ten.tea.2017.0419>
- [19] Tabatabaei, F.S., Tatari, S., Samadi, R. and Torshabi, M. (2016) Surface Characterization and Biological Properties of Regular Dentin, Demineralized Dentin, and Deproteinized Dentin. *Journal of Materials Science: Materials in Medicine*, **27**, Article No. 164. <https://doi.org/10.1007/s10856-016-5780-8>
- [20] Yuan, Z., Yuan, X., Zhao, Y., Cai, Q., Wang, Y., Luo, R., *et al.* (2021) Injectable GelMA Cryogel Microspheres for Modularized Cell Delivery and Potential Vascularized Bone Regeneration. *Small*, **17**, Article ID: 2006596. <https://doi.org/10.1002/sml.202006596>
- [21] Smith, A.J., Scheven, B.A., Takahashi, Y., Ferracane, J.L., Shelton, R.M. and Cooper, P.R. (2012) Dentine as a Bioactive Extracellular Matrix. *Archives of Oral Biology*, **57**, 109-121. <https://doi.org/10.1016/j.archoralbio.2011.07.008>
- [22] Gericke, A., Qin, C., Sun, Y., Redfern, R., Redfern, D., Fujimoto, Y., *et al.* (2010) Different Forms of DMP1 Play Distinct Roles in Mineralization. *Journal of Dental Research*, **89**, 355-359. <https://doi.org/10.1177/0022034510363250>
- [23] Chuang, S., Chen, Y., Ma, P.X. and Ritchie, H.H. (2022) Dentin Sialoprotein/Phosphophoryn (DSP/PP) as Bio-Inductive Materials for Direct Pulp Capping. *Polymers*, **14**, Article 3656. <https://doi.org/10.3390/polym14173656>
- [24] Ritchie, H. (2018) The Functional Significance of Dentin Sialoprotein-Phosphophoryn and Dentin Sialoprotein. *International Journal of Oral Science*, **10**, Article No. 31. <https://doi.org/10.1038/s41368-018-0035-9>
- [25] Ferracane, J.L., Cooper, P.R. and Smith, A.J. (2013) Dentin Matrix Component Solubilization by Solutions of pH Relevant to Self-Etching Dental Adhesives. *The Journal of Adhesive Dentistry*, **15**, 407-412.
- [26] Jiao, L., Xie, L., Yang, B., Yu, M., Jiang, Z., Feng, L., *et al.* (2014) Cryopreserved Dentin Matrix as a Scaffold Material for Dentin-Pulp Tissue Regeneration. *Biomaterials*, **35**, 4929-4939. <https://doi.org/10.1016/j.biomaterials.2014.03.016>
- [27] Liu, S., Sun, J., Yuan, S., Yang, Y., Gong, Y., Wang, Y., *et al.* (2022) Treated Dentin Matrix Induces Odontogenic Differentiation of Dental Pulp Stem Cells via Regulation of Wnt/ β -Catenin Signaling. *Bioactive Materials*, **7**, 85-97. <https://doi.org/10.1016/j.bioactmat.2021.05.026>
- [28] Yang, H., Li, J., Hu, Y., Sun, J., Guo, W., Li, H., *et al.* (2019) Treated Dentin Matrix Particles Combined with Dental Follicle Cell Sheet Stimulate Periodontal Regeneration. *Dental Materials*, **35**, 1238-1253.

- <https://doi.org/10.1016/j.dental.2019.05.016>
- [29] Jing, X., Xie, B., Li, X., Dai, Y., Nie, L. and Li, C. (2021) Peptide Decorated Demineralized Dentin Matrix with Enhanced Bioactivity, Osteogenic Differentiation via Carboxymethyl Chitosan. *Dental Materials*, **37**, 19-29. <https://doi.org/10.1016/j.dental.2020.09.019>
 - [30] Elfana, A., El-Kholy, S., Saleh, H.A. and Fawzy El-Sayed, K. (2021) Alveolar Ridge Preservation Using Autogenous Whole-Tooth versus Demineralized Dentin Grafts: A Randomized Controlled Clinical Trial. *Clinical Oral Implants Research*, **32**, 539-548. <https://doi.org/10.1111/clr.13722>
 - [31] Gao, X., Qin, W., Chen, L., Fan, W., Ma, T., Schneider, A., *et al.* (2020) Effects of Targeted Delivery of Metformin and Dental Pulp Stem Cells on Osteogenesis via Demineralized Dentin Matrix under High Glucose Conditions. *ACS Biomaterials Science & Engineering*, **6**, 2346-2356. <https://doi.org/10.1021/acsbiomaterials.0c00124>
 - [32] Um, I., Kim, Y., Park, J. and Lee, J. (2018) Clinical Application of Autogenous Demineralized Dentin Matrix Loaded with Recombinant Human Bone Morphogenetic-2 for Socket Preservation: A Case Series. *Clinical Implant Dentistry and Related Research*, **21**, 4-10. <https://doi.org/10.1111/cid.12710>
 - [33] Xu, X., Sohn, D., Kim, H., Lee, S. and Moon, Y. (2018) Comparative Histomorphometric Analysis of Maxillary Sinus Augmentation with Deproteinized Bovine Bone and Demineralized Particulate Human Tooth Graft: An Experimental Study in Rabbits. *Implant Dentistry*, **27**, 324-331. <https://doi.org/10.1097/id.0000000000000755>
 - [34] Li, P., Zhu, H. and Huang, D. (2018) Autogenous DDM versus Bio-Oss Granules in GBR for Immediate Implantation in Periodontal Postextraction Sites: A Prospective Clinical Study. *Clinical Implant Dentistry and Related Research*, **20**, 923-928. <https://doi.org/10.1111/cid.12667>
 - [35] Koga, T., Minamizato, T., Kawai, Y., Miura, K., I, T., Nakatani, Y., *et al.* (2016) Bone Regeneration Using Dentin Matrix Depends on the Degree of Demineralization and Particle Size. *PLOS ONE*, **11**, e0147235. <https://doi.org/10.1371/journal.pone.0147235>
 - [36] Gao, X., Qin, W., Wang, P., Wang, L., Weir, M.D., Reynolds, M.A., *et al.* (2019) Nano-Structured Demineralized Human Dentin Matrix to Enhance Bone and Dental Repair and Regeneration. *Applied Sciences*, **9**, Article 1013. <https://doi.org/10.3390/app9051013>
 - [37] Park, S., Kim, D. and Pang, E. (2017) Bone Formation of Demineralized Human Dentin Block Graft with Different Demineralization Time: *In vitro* and *in vivo* Study. *Journal of Cranio-Maxillofacial Surgery*, **45**, 903-912. <https://doi.org/10.1016/j.jcms.2017.03.007>
 - [38] Han, J., Jeong, W., Kim, M., Nam, S., Park, E. and Kang, H. (2021) Demineralized Dentin Matrix Particle-Based Bio-Ink for Patient-Specific Shaped 3D Dental Tissue Regeneration. *Polymers*, **13**, Article 1294. <https://doi.org/10.3390/polym13081294>
 - [39] Bi, F., Zhang, Z. and Guo, W. (2022) Treated Dentin Matrix in Tissue Regeneration: Recent Advances. *Pharmaceutics*, **15**, Article 91. <https://doi.org/10.3390/pharmaceutics15010091>
 - [40] Bakhtiar, H., Mazidi, A., Mohammadi-Asl, S., Hasannia, S., Ellini, M.R., Pezeshki-Modaress, M., *et al.* (2020) Potential of Treated Dentin Matrix Xenograft for Dentin-Pulp Tissue Engineering. *Journal of Endodontics*, **46**, 57-64.E1. <https://doi.org/10.1016/j.joen.2019.10.005>
 - [41] Li, R., Guo, W., Yang, B., Guo, L., Sheng, L., Chen, G., *et al.* (2011) Human Treated Dentin Matrix as a Natural Scaffold for Complete Human Dentin Tissue Regeneration. *Biomaterials*, **32**, 4525-4538. <https://doi.org/10.1016/j.biomaterials.2011.03.008>
 - [42] Li, H., Ma, B., Yang, H., Qiao, J., Tian, W. and Yu, R. (2021) Xenogeneic Dentin Matrix as a Scaffold for Biomineralization and Induced Odontogenesis. *Biomedical Materials*, **16**, Article 045020. <https://doi.org/10.1088/1748-605x/abfbbe>
 - [43] Chen, J., Liao, L., Lan, T., Zhang, Z., Gai, K., Huang, Y., *et al.* (2020) Treated Dentin Matrix-Based Scaffolds Carrying TGF- β 1/BMP4 for Functional Bio-Root Regeneration. *Applied Materials Today*, **20**, Article 100742. <https://doi.org/10.1016/j.apmt.2020.100742>
 - [44] Lan, T., Chen, J., Zhang, J., Huo, F., Han, X., Zhang, Z., *et al.* (2021) Xenoextracellular Matrix-Rosiglitazone Complex-Mediated Immune Evasion Promotes Xenogenic Bioengineered Root Regeneration by Altering M1/M2 Macrophage Polarization. *Biomaterials*, **276**, Article 121066. <https://doi.org/10.1016/j.biomaterials.2021.121066>
 - [45] Zhang, J., Lan, T., Han, X., Xu, Y., Liao, L., Xie, L., *et al.* (2021) Improvement of ECM-Based Bioroot Regeneration via *N*-Acetylcysteine-Induced Antioxidative Effects. *Stem Cell Research & Therapy*, **12**, Article No. 202. <https://doi.org/10.1186/s13287-021-02237-5>
 - [46] Han, X., Liao, L., Zhu, T., Xu, Y., Bi, F., Xie, L., *et al.* (2020) Xenogeneic Native Decellularized Matrix Carrying PPAR γ Activator RSG Regulating Macrophage Polarization to Promote Ligament-to-Bone Regeneration. *Materials Science and Engineering: C*, **116**, Article 111224. <https://doi.org/10.1016/j.msec.2020.111224>
 - [47] Sun, J., Li, J., Li, H., Yang, H., Chen, J., Yang, B., *et al.* (2017) *r*BHQ Suppresses Osteoclastic Resorption in Xenogeneic-

- Treated Dentin Matrix-Based Scaffolds. *Advanced Healthcare Materials*, **6**, Article ID: 1700127. <https://doi.org/10.1002/adhm.201700127>
- [48] Holiel, A.A., Mahmoud, E.M. and Abdel-Fattah, W.M. (2021) Tomographic Evaluation of Direct Pulp Capping Using a Novel Injectable Treated Dentin Matrix Hydrogel: A 2-Year Randomized Controlled Clinical Trial. *Clinical Oral Investigations*, **25**, 4621-4634. <https://doi.org/10.1007/s00784-021-03775-1>
 - [49] Xiong, Y., Shen, T. and Xie, X. (2022) Effects of Different Methods of Demineralized Dentin Matrix Preservation on the Proliferation and Differentiation of Human Periodontal Ligament Stem Cells. *Journal of Dental Sciences*, **17**, 1135-1143. <https://doi.org/10.1016/j.jds.2022.01.007>
 - [50] Karlsson, J.O.M. and Toner, M. (1996) Long-Term Storage of Tissues by Cryopreservation: Critical Issues. *Biomaterials*, **17**, 243-256. [https://doi.org/10.1016/0142-9612\(96\)85562-1](https://doi.org/10.1016/0142-9612(96)85562-1)
 - [51] Perry, B.C., Zhou, D., Wu, X., Yang, F.-C., Byers, M.A., Chu, T.-M.G., *et al.* (2008) Collection, Cryopreservation, and Characterization of Human Dental Pulp-Derived Mesenchymal Stem Cells for Banking and Clinical Use. *Tissue Engineering Part C: Methods*, **14**, 149-156. <https://doi.org/10.1089/ten.tec.2008.0031>
 - [52] Yan, W., Tenwalde, M., Øilo, M., Zhang, H. and Arola, D. (2018) Effect of Cryopreservation of Teeth on the Structural Integrity of Dentin. *Dental Materials*, **34**, 1828-1835. <https://doi.org/10.1016/j.dental.2018.10.004>
 - [53] Czochrowska, E.M., Stenvik, A., Bjercke, B. and Zachrisson, B.U. (2002) Outcome of Tooth Transplantation: Survival and Success Rates 17-41 Years Posttreatment. *American Journal of Orthodontics and Dentofacial Orthopedics*, **121**, 110-119. <https://doi.org/10.1067/mod.2002.119979>
 - [54] Wingenfeld, C., Egli, R.J., Hempfing, A., Ganz, R. and Leunig, M. (2002) Cryopreservation of Osteochondral Allografts: Dimethyl Sulfoxide Promotes Angiogenesis and Immune Tolerance in Mice. *The Journal of Bone & Joint Surgery*, **84**, 1420-1429. <https://doi.org/10.2106/00004623-200208000-00019>
 - [55] Ma, L., Makino, Y., Yamaza, H., Akiyama, K., Hoshino, Y., Song, G., *et al.* (2012) Cryopreserved Dental Pulp Tissues of Exfoliated Deciduous Teeth Is a Feasible Stem Cell Resource for Regenerative Medicine. *PLOS ONE*, **7**, e51777. <https://doi.org/10.1371/journal.pone.0051777>
 - [56] Li, Z., Zheng, C., Jiang, P., Xu, X., Tang, Y. and Dou, L. (2023) Human Digested Dentin Matrix for Dentin Regeneration and the Applicative Potential in Vital Pulp Therapy. *Journal of Endodontics*, **49**, 861-870. <https://doi.org/10.1016/j.joen.2023.04.010>
 - [57] Chen, J., Cui, C., Qiao, X., Yang, B., Yu, M., Guo, W., *et al.* (2017) Treated Dentin Matrix Paste as a Novel Pulp Capping Agent for Dentin Regeneration. *Journal of Tissue Engineering and Regenerative Medicine*, **11**, 3428-3436. <https://doi.org/10.1002/term.2256>
 - [58] Kim, Y., Bang, K., Murata, M., Mitsugi, M. and Um, I. (2017) Retrospective Clinical Study of Allogenic Demineralized Dentin Matrix for Alveolar Bone Repair. *Journal of Hard Tissue Biology*, **26**, 95-102. <https://doi.org/10.2485/jhtb.26.95>
 - [59] Chang, C., Lin, T., Wu, S., Lin, C. and Chang, H. (2020) Regeneration of Tooth with Allogeneous, Autoclaved Treated Dentin Matrix with Dental Pulpal Stem Cells: An *in vivo* Study. *Journal of Endodontics*, **46**, 1256-1264. <https://doi.org/10.1016/j.joen.2020.05.016>
 - [60] Yang, X., Ma, Y., Guo, W., Yang, B. and Tian, W. (2019) Stem Cells from Human Exfoliated Deciduous Teeth as an Alternative Cell Source in Bio-Root Regeneration. *Theranostics*, **9**, 2694-2711. <https://doi.org/10.7150/thno.31801>
 - [61] Ji, B., Sheng, L., Chen, G., Guo, S., Xie, L., Yang, B., *et al.* (2015) The Combination Use of Platelet-Rich Fibrin and Treated Dentin Matrix for Tooth Root Regeneration by Cell Homing. *Tissue Engineering Part A*, **21**, 26-34. <https://doi.org/10.1089/ten.tea.2014.0043>
 - [62] Li, B., Yu, F., Wu, F., Hui, T., A, P., Liao, X., *et al.* (2018) EZH2 Impairs Human Dental Pulp Cell Mineralization via the Wnt/ β -Catenin Pathway. *Journal of Dental Research*, **97**, 571-579. <https://doi.org/10.1177/0022034517746987>
 - [63] Yang, X., Wang, G., Wang, Y., Zhou, J., Yuan, H., Li, X., *et al.* (2019) Histone Demethylase KDM7A Reciprocally Regulates Adipogenic and Osteogenic Differentiation via Regulation of C/EBP α and Canonical Wnt Signalling. *Journal of Cellular and Molecular Medicine*, **23**, 2149-2162. <https://doi.org/10.1111/jcmm.14126>
 - [64] Li, Y., Zhou, W., Li, P., Luo, Q., Li, A. and Zhang, X. (2022) Comparison of the Osteogenic Effectiveness of an Autogenous Demineralised Dentin Matrix and Bio-Oss® in Bone Augmentation: A Systematic Review and Meta-Analysis. *British Journal of Oral and Maxillofacial Surgery*, **60**, 868-876. <https://doi.org/10.1016/j.bjoms.2022.03.009>
 - [65] Reis-Filho, C.R., Silva, E.R., Martins, A.B., Pessoa, F.F., Gomes, P.V.N., de Araújo, M.S.C., *et al.* (2012) Demineralised Human Dentine Matrix Stimulates the Expression of VEGF and Accelerates the Bone Repair in Tooth Sockets of Rats. *Archives of Oral Biology*, **57**, 469-476. <https://doi.org/10.1016/j.archoralbio.2011.10.011>
 - [66] Murata, M., Kabir, M.A., Hirose, Y., Ochi, M., Okubo, N., Akazawa, T., *et al.* (2022) Histological Evidences of Autograft of Dentin/Cementum Granules into Unhealed Socket at 5 Months after Tooth Extraction for Implant Placement. *Journal of Functional Biomaterials*, **13**, Article 66. <https://doi.org/10.3390/jfb13020066>