**Advances in Clinical Medicine** 临床医学进展, 2023, 13(5), 7141-7150
Published Online May 2023 in Hans. https://www.hanspub.org/journal/acm
https://doi.org/10.12677/acm.2023.135998

Hans汉斯

# 常见牙源性间充质干细胞及其在牙周再生的应用进展

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收稿日期：2023年4月7日；录用日期：2023年4月29日；发布日期：2023年5月9日

## 摘　要

**牙源性间充质干细胞是牙周组织再生工程的重要研究方向之一，主要包括DPSC、PDLSC，GMSC等。通过选择具有性能和应用优势的牙源性间充质干细胞作为种子细胞，充分发挥其诱导组织再生的能力，可在体内实现一定程度的牙周软硬组织修复效果。在牙周组织再生工程中，合适的生物医学材料常可作为载体负载牙源性间充质干细胞用于牙周炎导致的牙周支持组织缺损。本文将回顾不同牙源性间充质干细胞群体的特性及其在牙周组织再生中的应用进展。**

## 关键词

**牙源性间充质干细胞，牙周炎，组织再生**

# Common Odontogenic Mesenchymal Stem Cells and Their Application Progress in Periodontal Regeneration

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Received: Apr. 7th, 2023; accepted: Apr. 29th, 2023; published: May 9th, 2023

## Abstract

**Odontogenic mesenchymal stem cell is one of the important research directions of periodontal tissue regeneration engineering, mainly including DPSC, PDLSC, GMSC, etc. By selecting dental**

文章引用: 王文浩, 胡高辅, 王奥, 吴燕岷. 常见牙源性间充质干细胞及其在牙周再生的应用进展[J]. 临床医学进展, 2023, 13(5): 7141-7150. DOI: 10.12677/acm.2023.135998

**mesenchymal stem cells with performance and application advantages as seed cells and giving full play to their abilities to induce tissue regeneration, a certain degree of periodontal soft and hard tissue repair effect can be achieved *in vivo*. In periodontal tissue regeneration engineering, suitable biomedical materials can often be used as carriers to load dental mesenchymal stem cells for periodontal support tissue defects caused by periodontitis. This article will review the characteristics of different dental mesenchymal stem cell populations and their application progress in periodontal tissue regeneration.**

## Keywords

**Odontogenic Mesenchymal Stem Cells, Periodontitis, Tissue Regeneration**



## 1. 引言

牙周炎是一种以包括牙周膜组织、牙龈组织、牙槽骨组织在内牙周组织进行性破坏为主要特征的慢性炎症性疾病，在不及时干预的情况下，最终会造成牙齿脱落，从而影响患者咀嚼功能及美学效果[1]。牙周炎以牙菌斑生物膜为始动因子，与多种局部因素密切相关，并具有遗传易感性[2]。此外，牙周病与许多全身因素关系密切，可以相互影响、相互促进，如心脑血管疾病[3]、糖尿病[4]，及一些自身免疫性疾病如类风湿性关节炎等[5]。对于牙周炎的治疗，除了去除局部刺激因素、控制炎症的进展，从而控制牙周组织的持续破坏外，如何实现一定程度的牙周组织再生、进而保存牙齿是至关重要的。要达成这一目标，需要完成牙周组织各个组成部分的恢复，包括重建牙龈结缔组织、牙周膜、牙骨质和牙槽骨[6]。此外，牙周韧带纤维组的结构垂直插入牙骨质，并以规则的排列方式将其与牙槽骨相连，将牙齿固定于牙槽窝中，可承受和缓冲咀嚼力[7]。实现重建牙周组织的结构和功能的治疗目标需要同时实现软硬组织的再生，新生的牙槽骨必须通过牙周韧带与牙根面形成新附着[8]。牙周软硬组织复合体的复杂性、牙周韧带纤维结构和功能特点使得当前临床应用的常规牙周治疗技术所实现的促进牙周再生的效果有限，对牙周生物学的深入研究和生物支架材料的开发应用为牙周炎的治疗提供了新的思路。

干细胞生物学已成为再生医学领域的重要研究方向，自多能间充质干细胞(mesenchymal stem cells, MSC)在骨髓(bone marrow, BM)中被发现以来，各个组织中的间充质干细胞样细胞群体被提取出来，并以BMMSC 为金标准对其进行了多方面表征[9] [10] [11] [12]。牙源性间充质干细胞是一类牙及牙周组织来源的 MSC 样细胞群体，人们最早从牙髓组织中分离出了牙髓干细胞(dental pulp stem cells, DPSC) [13]，并随后从各个组织中分离出了来自脱落乳牙的干细胞(stem cells from exfoliated deciduous teeth, SHED) [14]，牙周韧带干细胞(periodontal ligament stem cells, PDLSC) [15]，来自根尖乳头的干细胞(stem cells from apical papilla, SCAP)等[16]。最近的研究还确定了更多来自牙囊和牙龈的牙齿组织来源的干细胞群[17] [18]。

这些不同的细胞群的扩增速率、多向分化能力均有不同，并已被用于各类动物模型的组织工程研究，评估其临床前应用的潜力[15] [19]。其中 PDLSC 因强大的再生分化、自我更新能力受到研究者们的广泛关注，并已在动物牙周炎模型和自体人 PDLSC 移植治疗牙周炎的研究中取得了良好的临床效果[6]。人们也从牙龈组织中分离出了间充质干细胞的独特亚群，除具有与其他组织来源 MSC 类似的增殖分化能力

外，其高度的增殖性和强大的免疫调节作用赋予了 GMSC 在各类疾病中强大的再生和治疗潜力[17] [20] [21]，且 GMSC 采集于牙龈组织从而易于获得。PDLSC 和 GMSC 的研究可能对于组织工程学和再生医学的发展具有重要意义。本文将综述几种常见牙源性间充质干细胞包括 DPSC、PDLSC、GMSC 的分离、表征及在牙周组织再生中的应用进展。

## 2. 牙源性间充质干细胞的分离及表征

### 2.1. 牙源性间充质干细胞的分离

各种类型来源的牙龈组织中均可提取出 GMSC，如人第三磨牙拔除时牙颈部周围的牙龈组织[22]、牙周健康患者因牙周手术切除的牙龈组织等[23]。以磷酸盐缓冲盐水(phosphate-buffered saline, PBS)和胶原酶初步处理后可以将牙龈组织的结缔组织层和上皮层进行剥离，随后将结缔组织切成小块，种植在培养皿中(组织块－酶消化法)。在因正畸拔除的前磨牙、第三磨牙牙根中三分之一段可分离到较纯的牙周膜组织。牙髓组织可以以注射器针头自牙髓腔中刮取[24]。牙周膜组织和牙髓组织同样以组织块－酶消化法植入培养皿，等待原代细胞爬出[25]。上述几种细胞均可在 3~7 天左右的时间贴壁爬出，外观呈纺锤形成纤维细胞样。国际细胞治疗学会(International Society for Cellular Therapy, ISCT)提出了定义多能间充质干细胞的最低标准：1. 塑料粘附性：在标准培养瓶中的标准培养条件下培养时，细胞必须具有塑料贴壁性；2. 特异性表达抗原：至少 95%的 MSC 群体必须表达免疫表型标志物 CD105、CD73 和 CD90，少于 2%的细胞群体表达标志物 CD45、CD34、CD14 或 CD11b、CD79$\alpha$、CD19 等，干细胞标志物也可在不同组织中分离培养的成纤维细胞表达，但其不具有多向分化能力；3. 多向分化潜力：MSC 细胞群体必须在标准体外分化条件下产生至少三种细胞谱系，成骨、成软骨、成脂分化潜力[26]。

### 2.2. 牙源性间充质干细胞增殖及分化能力

GMSCs 比 BMSC 表现出更强的增殖能力，人 GMSC 的平均种群倍增时间为 39.6 ± 3.2 小时，远低于 BMSC (80.4 ± 1.2 小时)，在生长增殖过程中，GMSC 的镜下形态可以保持稳定，在长期的传代培养中可以始终保持 MSC 的特性及端粒酶活性[20]。GMSC 的间充质干细胞表面标志物(如 CD73，CD90，CD105，SSEA-4 和 STRO-1)呈阳性表达，但 CD34 和 CD45 等造血干细胞标志物呈阴性表达。其多能分化能力不仅包括向中胚层三系的分化潜力，即成骨、成软骨和成脂肪方向的分化[27]，还具有转化为外胚层和内胚层细胞谱系的潜力，如牙源性细胞[28]、内皮细胞[27]、神经细胞[29]和角质形成细胞[30]等。此外，GMSC 已被证明在小鼠动物模型中具备成骨和成结缔组织的能力[31]。因此，来源于人牙龈组织的干细胞的特性满足 ISCT 定义间充质干细胞的最低标准。

许多研究表明 PDLSC 显示出优越的增殖能力，其增殖能力优于 BMSC 和 DPSC [32]，弱于 GMSC [25]，且年轻供体来源的 PDLSC 比老年供体来源的 PDLSC 表现出更强的增殖活性和集落形成能力[33]，即细胞的衰老会影响 PDLSC 的增殖能力。多种因素可能对 PDLSC 的增殖活性产生影响，如白细胞介素-11 (interleukin-11, IL-11)、血管紧张素 II (angiotensin-II, Ang-II)等多种信号因子[34]、缺氧条件[35]、Rho-激酶信号等[36]通过丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)信号通路诱导 PDLSC 的增殖和分化。PDLSC 也具有分化成各种类型中胚层干细胞谱系的能力，包括成骨细胞样细胞、成脂肪细胞样细胞等[14]，及分化为神经细胞、少突胶质细胞、星形胶质样细胞等其他谱系细胞的能力[37]。PDLSC 和 GMSC 在体外成骨诱导培养基中培养时，均可形成矿化的细胞外基质，茜素红染色呈阳性，但定量结果显示 PDLSC 形成了更多矿化结节。在 ALP 染色实验和成骨相关基因如 OCN、RUNX-2 和 COL-1 的检测中，PDLSC 的表达水平明显高于 GMSC，因此 PDLSC 的成骨潜力优于 GMSC [38] [39]。

DPSC 也可表现出间充质干细胞特性，在牙髓组织中的存在比例较高[40]。与 BMSC 相比，DPSC 同样具有更高的增殖率(+30%)，这归因于细胞周期蛋白依赖性激酶-6 和 DPSC 中胰岛素样生长因子等细胞周期介质的表达[12]，但增殖能力弱于 PDLSC [41]。DPSC 能够在体外分化为成牙本质细胞、成骨细胞、神经源性细胞、成脂性细胞、肌源性细胞、成软骨细胞和黑色素细胞等细胞谱系，向小鼠动物模型中移植后可在体内环境下形成类似于牙本质和骨骼的矿化基质[12] [13] [42]-[47]。与 PDLSC 相比，DPSC 可以向成牙本质细胞方向分化，并较明显地表达成牙本质细胞表面标志物，而 PDLSC 的该标志物未见明显表达[41]。在成骨分化方面，不同于 PDLSC 显著的成骨分化性能，DPSC 的成骨分化 Notch 通路受到抑制，使得其成骨分化能力较差[48]。因此，DPSC 和 PDLSC 在生物学和治疗潜力方面有所不同。

### 2.3. 免疫调节能力

除多能分化能力外，GMSC 还可通过调节体外和体内各种先天性和适应性免疫细胞的表型和活化而具有强大的免疫调节和抗炎功能[22]。GMSC 能够将巨噬细胞极化为 M2 表型，促进抗炎细胞因子 IL-10 的分泌；抑制 M1 巨噬细胞极化，进而抑制促炎细胞因子 TNF-$\alpha$ (肿瘤坏死因子-$\alpha$，tumor necrosis factor-$\alpha$，TNF-$\alpha$)的分泌[49]，从而对巨噬细胞的表型和活化进行有效的免疫调节。GMSC 可降低肥大细胞(Mast cells, MC)表面标志物 CD117 的表达，抑制 MC 的主要促炎细胞因子 TNF-$\alpha$ 和 IL-1 的合成；GMSC 显著抑制成熟树突状细胞(Dendritic cells, DC)标志物 CD11c 和 CD80 的表达，并通过释放促炎细胞因子 IL-12 从而产生前列腺素 E2 (prostaglandin E2, PGE2) [50]。因此，GMSC 对先天免疫细胞，特别是巨噬细胞，树突状细胞和肥大细胞具有强大的免疫调节作用，但在人体不同病理条件，尤其是牙周炎中 GMSC 调节先天性免疫的详细作用机制仍需进一步研究。GMSC 可以通过多种机制抑制 T 细胞增殖活化并诱导 T 细胞凋亡[51]，可直接抑制 B 细胞活化和抗原呈递能力(CD80 和 CD86) [52]，还可以有效抑制外周血单核细胞(Peripheral Blood Mononuclear Cells, PBMC)的增殖和活化[53]。GMSC 对适应性免疫细胞的不同亚型同样具有有效调节作用。在人体牙周炎环境中，GMSC 调节适应性免疫的详细作用机制仍需进一步研究。

同属于间充质干细胞范畴，PDLSC 和 DPSC 的免疫调节作用在某些方面与 GMSC 相似，比如 PDLSC 同样可以抑制 PBMC 的增殖，活化的 PBMC 分泌干扰素-$\gamma$ (interferon-gamma, IFN-$\gamma$)，IFN-$\gamma$ 处理的 PDLSC 通过上调吲哚胺 2,3-双氧酶-1 (indolamine 2,3-dioxygenase-1, IDO-1)抑制 PBMC 生长[54]。GMSC 对 PBMC 的免疫调节能力也取决于 IFN-$\gamma$ 信号通路。DPSC 同样可以抑制同种异体 PBMC 的增殖。二者共培养后，PGE2、IL-6 和 TGF-$\beta$ 抗炎细胞因子水平升高，发挥免疫调节作用。此外，有研究表明在 DPSC 分化过程中，尽管未分化的 DPSC 和成骨方向分化的 DPSC 在某些细胞因子的产生方面存在一些差异，但分化后的细胞仍能够维持 IDO、HLAG 和 HGF 等免疫调节分子的表达，并且抑制植物血凝素(phytohaemagglutinin, PHA)诱导的淋巴细胞的增殖和分化，从而保持其免疫调节活性[55]。

在我们先前的研究中，GMSC 和 PDLSC 的体外再生和分化能力存在差异。尤其 PDLSC 在成骨分化方面优于 GMSC，这是我们在动物实验中关注的焦点。然而，许多研究表明 GMSC 在一些自身免疫和炎症疾病中表现出了强大的免疫调节和抗炎作用，且受炎症环境对细胞性能表达的影响更小[56] [57] [58] [59] [60]。因此，我们使用 GMSC 和 PDLSC 进行牙周组织再生的动物实验效果未见明显差异，它们都是牙周组织工程的优秀候选种子细胞。GMSC 具备更容易获得、体外增殖时间更短的优势，其再生应用潜力值得更多的关注和探索。

## 3. 牙周组织再生中的应用

尽管各型牙源性间充质干细胞的分化潜力已被充分验证，但要想实现真正的牙周组织再生需要将细胞或细胞衍生物输送至牙周病变区域，并保持一定时间的生物活性。因此，生物支架材料在干细胞促牙

周再生的应用中十分重要，理想的支架材料应具有以下特性：1. 具有保存细胞的能力，并可提供细胞生存所必须的空间结构，细胞可进行生长增殖分化所必须的营养、气体交换等；2. 支架材料应具备良好的生物相容性和低细胞毒性；3. 具有自然降解性能，免疫原性低[61]。近年来以实现牙周组织再生的目标，各类新型生物材料被设计制作，纳米复合类材料如静电纺丝纤维[62]，刚性支架类材料如硅灰石复合材料等[63]，及各类水凝胶材料如纤维加强类水凝胶[64]、具有热敏性的壳聚糖水凝胶[65]、高刚度转谷氨酰胺酶交联水凝胶等[66]，各种类型的材料均被证明对牙周再生有不同程度的促进效果。

科学家们对 GMSC 在牙周再生领域的探索并不充分，但仍证明了基于 GMSC 的再生治疗在解决牙周炎问题的可能性。通过在小鼠中使用已建立的牙周炎模型，Sun W 和 Liu X 等研究者发现，GMSC 的全身应用有助于牙槽骨高度增加和新骨形成，对促进牙周组织再生有积极意义[67] [68]。在进一步的研究中，GMSC 细胞膜片用于比格犬模型的牙周再生，移植的 GMSC 显著增强了受损牙周组织包括牙槽骨、牙骨质和牙周韧带的再生[69]。基于透明质酸的 IL-1 释放水凝胶与 GMSC 的结合在猪实验性牙周炎模型中显示出了显著的牙周再生潜力[70]。此外，GMSC 衍生的条件培养基和外泌体的局部应用也分别对大鼠[71]和小鼠[57]的牙周炎表现出一定的治疗效果。

相比于 GMSC，人们对 PDLSC 促牙周再生作用的研究则更为丰富，2004 年，首次有科学家使用羟基磷灰石支架材料承载 PDLSC 进行了动物实验，移植入小鼠牙周组织缺损模型，其结果验证了 PDLSC 对牙周骨缺损和结缔组织修复具有一定促进作用；以羟基磷灰石/磷酸三钙(hydroxyapatite/tricalcium phosphate, HA/TCP)颗粒为支架承载 PDLSC，将其移植到免疫缺陷小鼠的背部皮下。在移植 8 周后成功生成了牙周韧带、牙骨质及骨组织，完成了体内条件下 PDLSC 生成牙周组织各个部分能力的验证[72]；还有研究在狗的上颌尖牙和第一磨牙将 PDLSC 进行了自体移植，通过手术形成牙周缺损，取得了优越的骨再生效果，但牙周韧带和牙骨质形成有限，说明在体内炎症环境下，实现复杂的牙周复合体的再生仍存在较大困难[73]；为适应牙齿及牙周解剖结构的复杂性，快速发展的 3D 打印技术为牙周再生医学提供了新的思路，可通过制作多层次、形状灵活的支架材料负载 PDLSC 实现牙周再生的诱导[74]。

更多关于 DPSC 促再生的研究集中在骨再生和牙髓－牙本质复合体领域，Petridis 等人评估了人牙髓细胞复合透明质酸水凝胶支架在大鼠颅骨缺损中的成骨作用[75]；另有研究将牙髓干细胞 DPSC 接种在静电纺丝聚 $\varepsilon$-己内酯/明胶支架上，无论是否添加纳米羟基磷灰石，均可上调小鼠特定牙源性基因的表达，增强了 DPSC 在体内环境下向成牙本质细胞样表型的分化[76]。在牙周组织工程方面，已有研究证明了将 DPSC 来源的外泌体掺入壳聚糖水凝胶，可以通过抑制牙周炎症和调节免疫反应来加速小鼠牙周炎模型中牙槽骨和软组织的愈合[77]。

几种牙源性间充质干细胞的细胞衍生物和各型载细胞支架材料的应用取得了丰硕的再生成果，以牙源性间充质干细胞特别是 GMSC、PDLSC 的手段治疗牙周组织缺损的安全性也已被充分验证。但当前仍缺乏大样本量的多中心临床试验和随机对照研究，验证牙源性间充质干细胞治疗牙周炎的安全性、有效性。

## 4. 总结

综上所述，GMSC、PDLSC 和 DPSC 均为具有自我更新、多向分化及一定免疫调节能力的间充质干细胞，但特性有所差异，均可通过体外组织块法分离提取。牙源性间充质干细胞尤其是 GMSC、PDLSC 是优秀的组织工程和再生治疗的种子细胞，在牙周组织再生的应用方面仍需更多更充分的研究。

## 基金项目

浙江大学科研项目资助(项目编号：XY2021029)。

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