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三维生物打印在牙周组织工程的研究进展及展望

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摘　要

牙周炎是一种慢性炎症性疾病，导致牙周组织尤其是骨组织的破坏，是成年人失牙的首位原因。三维生物打印作为21世纪初的新兴技术，包裹细胞的生物墨水可通过模拟发育过程中组织的自组装促进再生，在组织工程中的应用前景巨大。3D打印技术带来了牙周再生方法的重大创新，三维打印不仅可以打印生物相容性膜和支架，还可以打印复杂3D功能组织中的活细胞和支持部件。本文综述了牙周再生领域相关的种子细胞、生长因子、生物支架，讨论了其目前面临的挑战，同时对其在牙周再生领域的应用进行了展望。

关键词

三维生物打印，牙周再生，生物支架，种子细胞，生长因子

Progress and Prospect of 3D Bioprinting in Periodontal Reconstruction

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Abstract

Periodontitis is a chronic inflammatory disease that leads to the destruction of periodontal tissue,

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especially bone tissue, and is the first cause of tooth loss in adults. As an emerging technology in the early 21st century, three-dimensional (3D) bioprinting can promote regeneration by simulating the self-assembly of tissues during development, which has great application prospects in tissue engineering. 3D printing technology has led to major innovations in periodontal regeneration methods, allowing the printing not only of biocompatible membranes and scaffolds, but also of living cells and supporting components in complex 3D functional tissues. This paper reviews the research progress of the periodontal regeneration, which mainly refers to seed cells, growth factors and biological scaffold, and discusses their current challenges, applications in the field of periodontal regeneration.

Keywords

Three-Dimensional Bioprinting, Periodontal Regeneration, Biological Scaffold, Seed Cell, Growth Factor

1. 前言

牙周炎是一种多因素引起的牙周组织炎症性疾病[1] [2]，一项流行病学调查表明，世界上超过 50%的成年人患有牙周病。此外，牙周病的发病率随着时间的推移而增加[3]。牙周病的发病机制涉及宿主免疫应答、牙周袋内微生物菌落之间复杂的相互作用以及吸烟和遗传等其他因素[4]。此外，牙周炎还与许多其他全身性疾病有关[5]，如心血管疾病、肥胖、癌症、糖尿病和类风湿关节炎[6]-[11]。这种病理的特点是牙周附着逐渐丧失。随着时间的推移牙槽骨的破坏，可能导致牙齿脱落。严重影响患者的生理功能和生活质量[12]。三维生物打印技术作为组织工程和再生医学的一种新兴方法，为高效解决制备功能化人体组织的难题提供了新思路。由于其可自动化操作和高通量的优势，近年来，在牙周再生领域得到了很好的应用。本文主要对 3D 打印技术的发展和牙周组织工程的生物支架、种子细胞和生物活性因子两部分进行综述。

2. 3D 打印技术概述

三维打印技术早在 1986 年就被开发出来。近十年来，3D 打印技术已广泛应用于再生医学、解剖模型制作、手术指南制作以及药物配方等不同医学领域[13] [14] [15]。近年生物打印的概念被明确定义为“使用计算机辅助、对具有特定二维或三维组织的活体和非活体材料进行图案化和组装的过程，以生产用于再生医学、药代动力学和基础细胞生物学等研究的生物工程化结构”[16]。3D 打印在解决牙周组织复杂的分层组织(包括牙龈、牙骨质、牙周膜和牙槽骨)的需求方面特别有价值[17] [18]。采用多相生物材料构建的方法可以再现因创伤、慢性感染或手术切除而破坏的牙齿支撑组织的结构完整性。一系列的连续研究旨在开发 3D 打印仿生复合杂化聚合物支架来复制牙本质－牙周膜－骨界面[19]。

3. 三维生物打印在牙周组织工程的研究

3.1. 生物支架

用于牙周再生的支架，无论是单相还是多相，都可以使用天然聚合物、合成聚合物、生物陶瓷或金属等各种材料进行 3D 打印[20] [21]。Lee [21]等人开发了具有时空递送蛋白质的多相区域特异性微支

架，用于牙周组织的综合再生。采用三维打印技术，将聚乳酸羟基磷灰石支架分三个阶段制备。对于现有支架材料的改性，也是目前 3D 打印技术在牙槽骨再生领域的研究热点。比如，脂肪族聚酯由于其成本低、保质期强以及能大量生产的优势一直是研究最多的合成聚合物，包括聚己内酯(PCL)、聚乳酸(PLA)、聚乙醇酸(PGA)及聚乳酸－羟基乙酸共聚物(PLGA)。然而，脂肪族聚酯在降解时会释放出酸性副产物，可导致组织坏死和支架失效[22]。它们通常与生物陶瓷结合使用，以增强构建物的生物活性，并通过提高支架的整体 pH 值来中和酸性副产物。最新发现，当聚酯与镁和镁合金结合时，也可以抵消酸性副产物[23]。

3.2. 种子细胞

牙周袋内的炎症可改变病理性牙周组织的细胞生物学。牙周组织一旦受损，其再生能力就有限，而再生能力依赖于间充质干细胞。间充质干细胞负责组织稳态，作为可再生祖细胞的来源。此外，到目前为止的研究证实，将牙周干细胞移植到牙周缺损中，不会产生不良的免疫或炎症后果。因此，牙周再生可通过局部来源的可再生祖细胞成功募集到病变部位以维持组织稳态，随后分化为牙周膜、牙骨质和成骨细胞[24]。可通过向缺损部位输送干细胞成功形成新的牙周膜[25]，包括输送牙周膜干细胞(PDLSCs)、骨髓间充质干细胞(BMMSCs)、脂肪间充质干细胞(ADSCs)和诱导多能干细胞(iPSCs) [26] [27]。Ma [28]等人通过控制明胶甲基丙烯酸酯(GelMA)和聚乙二醇二甲基丙烯酸酯(PEG)的体积比，生成具有细胞外基质组分梯度的纳米级负载三维细胞水凝胶阵列。用来筛选人牙周膜干细胞(PDLSC)对细胞外基质的反应。

此外，有学者通过纤维增强水凝胶方法将聚己内酯(PCL)以三维结构的形式与具有生物活性的负载无定形磷酸镁的明胶甲基丙烯酸酯(GelMA)水凝胶相结合。该复合材料利用无定形磷酸镁和 PCL 网有效控制了机械性能并增强成骨潜力。这一创新在引导骨再生(GBR)领域显示出巨大的前景。研究结果表明，结合 PCL 网格创建使用熔融电子书写，有助于阻止水凝胶的降解，防止软组织的渗透。此外，PCL 网状物作为一种机械屏障，促进缓慢移动的祖细胞参与骨再生并随后转化为成骨细胞[29] [30] [31] [32]。与基于细胞的治疗策略相比，基于生物材料的方法为大量内源性组织再生提供了相对直接和可靠的支持手段。因此，内源性再生技术已经成为一种预算友好、高效、安全的临床患者治疗方法。

3.3. 生物活性因子

生物活性分子在牙周再生中起着关键作用。目前研究发现促进牙周再生的生物活性因子包括血小板源性生长因子(PDGF) [33]、釉基质蛋白衍生物(EMD) [34]和骨形成蛋白(BMPs) [35]等。这些分子在再生过程的各个方面起着重要的调节作用。它们影响前体细胞向特定牙周组织的分化，刺激原位干细胞迁移到受损部位，并吸引免疫细胞调节炎症反应，从而促进牙周组织的再生[24] [30] [36]。Nagayasu-Tanaka [37]等人通过将牙周再生药物成纤维细胞生长因子(FGF-2)与生物可吸收支架碳酸磷灰石联合使用用于研究牙周再生。研究发现其结合物可以促进新骨形成和支架的替换，诱导的新牙周组织类似于自发愈合形成的成熟过程。

4. 前景与展望

由于牙周器官的复杂性质和结构，牙周再生涉及到高度的复杂性。牙龈、牙周韧带、牙槽骨和牙骨质的协调对牙周组织的完整再生至关重要。此外，牙周系统容易受到微生物菌群的影响，也容易受到牙周病的局部和全身危险因素的影响。然而三维生物打印目前在牙周膜重建方面仅有体外研究，这应该与牙周膜复杂精细有序结构、口腔的湿润环境、有限的操作空间以及持续的咀嚼压力相关。将体外和体内模型转化为人类受试者的临床模型是目前的主要挑战。此外，目前的研究很少关注抗菌和免疫调节策略。

抗菌药物和具有免疫调节能力的生物材料的整合是一个未来可以研究的方向。

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