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无支架3D工程化脂肪的构建

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摘　要

脂肪组织工程是创伤、肿瘤切除后软组织缺损潜在的治疗方法，大多数方法都依赖于使用外源性3D支架来再生脂肪组织。近年来，随着生物制造技术的不断发展，无支架3D工程化脂肪的制备已成为一种备受关注的生物制造方法。由于无支架组织结构不需要细胞粘附在外源材料上并且只涉及细胞和细胞衍生基质，因此无支架组织工程比基于支架的方法提供了许多优势：1) 没有引入任何外源性杂质；2) 小分子扩散、细胞之间的信号传递、细胞迁移不受移植后的影响；3) 仅依赖细胞产生基质。本文综述了脂肪组织工程的三种主要无支架方法：自组装技术，生物反应器和磁悬浮技术，详细阐述了近年来的研究进展及优缺点。

关键词

脂肪，组织工程，无支架，自组装，生物反应器，磁悬浮

Construction of Scaffold-Free 3D Engineered Adipose

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Abstract

Adipose tissue engineering is a potential treatment for soft tissue defects after trauma and tumor resection, most of which rely on the use of exogenous 3D scaffolds to regenerate adipose tissue. In recent years, with the continuous development of biomanufacturing technology, the preparation of stent-free 3D engineered fats has become a kind of biomanufacturing method. Since stentless

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tissue structures do not require cell adherence to foreign materials and involve only cells and cell-derived matrices, stentless tissue engineering offers many advantages over stent-based approaches: 1) No exogenous impurities are introduced; 2) The diffusion of small molecules, signal transmission between cells and cell migration were not affected by transplantation; 3) Matrix production depends only on cells. In this paper, three main stent-free methods for adipose tissue engineering, self-assembly, bioreactor and magnetic levitation, are reviewed. The research progress, advantages and disadvantages in recent years are described in detail.

Keywords

Adipose, Tissue Engineering, Scaffold-Free, Self-Assembly, Bioreactors, Magnetic Levitation

1. 引言

脂肪组织工程是一种能够开发自体移植物的新兴方法，可以有效治疗因创伤、肿瘤切除导致的软组织缺损。几个研究小组利用常用的 3T3-L1 小鼠前脂肪细胞系[1] [2]、大鼠前脂肪细胞[3] [4]或骨髓间充质干细胞[5] [6] [7]开创了脂肪组织工程的先河。通过构建类似于自然脂肪组织的人工组织，可以为人体提供更好的医学治疗手段。

传统的二维细胞培养技术无法提供细胞与细胞之间、细胞与基质之间的三维相互作用。三维(3D)体外培养模型可以更好地模拟脂肪组织在体内复杂的生理环境[8]，并且可明显改善脂肪的形成和分化[9] [10]。基于支架的 3D 培养在细胞培养中起到承载和定位细胞的作用，也为再生组织提供了必须的机械强度和支持。各种天然或合成的生物可降解聚合物支架与动物或人类来源的脂肪前体细胞结合进行了试验。已经报道了纤维蛋白[11]，胶原海绵[12] [13]，透明质酸基支架[14]，海藻酸盐珠[15]，聚乳酸－羟基乙酸共聚物[7] [16]，涂有胶原的聚四氟乙烯网[17]，以及可脱水和复水以获得所需形状的海藻酸盐或透明质酸基水凝胶[12]。支架材料的选择和制备对 3D 细胞培养的成功非常重要，但它们也会引起巨噬细胞引发的异物反应，导致炎症和毒性，并且由于降解非常缓慢，还会干扰移植部位组织的再生。无支架 3D 细胞培养技术是一种仅由细胞及其自身分泌的基质即可构建三维组织的技术。该技术主要利用细胞本身的自组装能力，将细胞聚集成三维结构，从而实现组织构建。与传统的支架材料相比，无支架 3D 细胞培养技术具有更好的生物相容性、较低的毒性和更高的组织特异性，并且能够实现更为真实、可控和可重复的组织构建。因此，无支架 3D 细胞培养技术在脂肪组织工程方面的应用前景广阔。

大多数细胞可以创建自组装的 3D 结构，因为它们倾向于形成簇。这种自然的聚集倾向使细胞能够分泌并形成细胞外基质(ECM)成分，从而减轻了在系统中使用支架的需要。无支架 3D 细胞培养技术在脂肪组织工程方面的应用主要集中在两个方面：脂肪细胞的培养和多能干细胞的分化。在脂肪细胞的培养方面，无支架 3D 细胞培养技术被用来培养成熟的脂肪细胞，从而构建更为真实的脂肪组织工程。在这种方法中，细胞自发地形成 3D 结构，类似于体内的组织结构。这种方法不仅可以模拟脂肪组织的生理状态，还可以实现多次细胞增殖和分化，从而进一步提高组织工程的成功率。此外，无支架 3D 细胞培养技术也被用于多能干细胞的分化。多能干细胞是一种能够分化成多种不同类型细胞的细胞，可以通过特定的培养条件转化成脂肪细胞。无支架 3D 细胞培养技术可以提供一个更为真实的生理微环境，模拟

细胞在体内的生长和发育过程，从而增强多能干细胞向脂肪细胞分化的能力。已建立的脂肪组织工程方案，如悬滴法[18]、生物反应器[19]和磁悬浮技术[20]使得能够通过细胞的这种自组装行为形成球体。

2. 自组装技术

自组装技术是生成 3D 脂肪组织最简单的方法。基于传统的悬浮滴定培养法，Bohrnsen 等人利用干细胞的聚集特性，发明了间充质微球(MMS)培养系统[21]，实现了 3D 细胞－细胞相互作用过程中的成脂分化。与单层培养相比，MMS 培养提高了来源于小鼠肾周脂肪组织(PAT)、纵隔间质组织(MST)和小鼠骨髓(BM)的细胞成脂分化能力。功能性脂肪类器官模型系统通过悬浮滴定培养技术使 ASCs 自聚集成球体，随后转移到琼脂包被的细胞培养皿中，以避免细胞的贴壁和解聚。利用脂肪生成激素混合物诱导其脂肪细胞分化过程中，脂肪类器官的大小显著增加。染色发现在分化的细胞中有大量的单室和多室脂肪沉积，这表明 ASC 高效地分化为成熟的脂肪细胞。在脂肪类器官形成过程中，关键脂肪生成和脂肪细胞标志物 C/EBP-β，PPAR-γ，FABP4 过表达[22]。Baraniak 使用强制聚集技术形成不同大小的间充质干细胞(MSC)球体，并在分化培养基中悬浮培养维持较长时间后表现出成脂倾向的组织学标记[23]。Wang 将 hADSC 悬浮培养形成细胞聚集体以助于维持细胞存活。无论原始细胞密度如何，大多数聚集体的直径都在 50~200 μm 的范围内。此外，组织形态学和基因表达分析结果表明，与单层培养相比，悬浮培养中诱导 hADSC 更有效地分化为脂肪细胞[24]。Shen 等人开发了一个无支架的多功能 3D 脂肪细胞培养平台，对来自各种来源的人和鼠脂肪细胞模型的无支架球状体培养进行了优化。准确地模拟原代人前脂肪细胞向脂肪细胞的分化。多组学分析和功能测试表明，3D 脂肪细胞培养具有成熟的分子和细胞表型，类似于新分离的成熟脂肪细胞[25]。虽然 3D 球状体优于传统的二维单层培养的人类脂肪来源干细胞(hASCs)，但在增强其成脂分化和最大限度地减少培养过程中生理相关的脂肪球体损失的方法上尚未达成共识。相关研究证明在超低附着静态培养和悬浮培养方法中，球体合并形成更大的球体。而弹性蛋白样多肽－聚乙烯亚胺涂层对 hASC 球体尺寸和数量的保留效果最好[26]。

Vermette 等人从抽脂或切除的脂肪中提取人基质细胞，并使用适应的“自组装”培养，诱导脂肪分化同时补充抗坏血酸，发现脂肪基质细胞在抗坏血酸刺激下分泌和组织丰富的内源性 ECM，其中充满脂质的脂肪细胞嵌入富含纤维连接蛋白以及胶原 I 和 V 的 ECM 中。这种变化导致产生可操作的薄片，继而可组装成更厚的脂肪组织，形成一种天然的“生物材料”[27] [28]。Verseijden 将人脂肪组织间充质基质细胞(ASC)和人脐静脉内皮细胞(HUVEC)结合在球体共培养物构建了直径达 600 μm 的 ASCs 球体组成的脂肪组织[29]。这种自组装技术的适应性不仅与向脂肪细胞的分化相容，而且抗坏血酸补充对促进人基质细胞的脂肪分化有积极的影响。在功能水平上，重建的脂肪组织表达脂肪细胞相关的转录本和脂肪组织典型的分泌脂肪因子，如瘦素。扫描电镜观察发现，这些新型脂肪替代品与天然脂肪十分相似，并表现出白色脂肪组织的主要生物学特征。因此，这种组织工程方法的优点是生产一种功能性的完全天然的“生物材料”，有潜力用作体外特定代谢分析的脂肪替代品，以及用于重建和美容手术的自体软组织。

利用一些类似蜂窝或锥形装置设备还可通过打印微图案井来实现球体的成形[30] [31]。用于成簇培养的锥形模板(TASCL)装置有效地创造了一个体外微环境，装置中每个微井底部的超低细胞附着表面性质防止细胞粘附。合成的人类 ASC 球体是“类脂肪微组织”，完美地形成球形聚集体。TASCL 装置以与其它细胞支架相同的方式发挥作用，促进人 ASC 的成脂分化。基于纳米技术的纳米培养板(NCP)表面是呈蜂窝状排列直径为 2~3 μm 的不均匀微结构。UET-13 间充质祖细胞在 NCP 板内形成粘附球。NCP 的表面材料是无粘附性的合成树脂，但微细的结构使细胞能够粘附在有细胞突起的板上[32]。这种细胞－板的粘附比细胞－细胞的粘附弱。更低的细胞－板的粘附会促进球体的形成。当人骨髓间充质干/祖细胞(MPCs)用 NCP 进行 3D 培养时，它们迅速形成粘附球体，脂肪分化也比二维培养更快的甘油三酯积累。

此外，在 3D 培养的脂肪形成过程中，观察到快速而强烈地诱导脂肪细胞特异性基因表达，如 PPAR-γ、C/EBP-α、aP2 和脂联素。这些结果表明，该 3D 培养系统适合于人 MPCs 向成脂谱系的分化，可以应用于无异种条件下的脂肪组织工程。

3. 生物反应器

生物反应器的应用也为延长培养时间和高产出量的组织工程提供了一种无支架的方法。各种类型的生物反应器可被利用，如中空纤维生物反应器[33] [34]、连续搅拌槽生物反应器[35]、旋转壁生物反应器[36]、Couette-Taylor 生物反应器[37]和剪切流灌注生物反应器[38]。然而，生物反应器中的连续旋转会导致细胞受到长时间的剪切应力[39]。

大多数灌注生物反应器受限于样品室和/或流动通道的几何形状，常对生物材料施加不均匀的流动应力和剪切力。急性膨胀或非旋转对称的几何形状导致在样品室的周边产生不规则的流速区域[40]。这意味着将培养基流量调节到平均条件会导致一些样品区暴露于很小的流动应力和剪应力，而另一些样品区暴露于过度的流动应力和剪应力，这可能会影响细胞的增殖和分化[41] [42]。为了规避这一限制，Gordian 等人创建灌注生物反应器，即使填充了支架材料，生物反应器中灌注室的设计保证了在整个样品中均匀的流速、压力和剪切应力[43]。

几个研究小组使用动态 3D 灌注生物反应器来扩大和分化细胞[33] [34] [44]。与直接灌注和旋转壁悬浮相比，基于多室结构的半透性中空纤维交织的生物反应器设计具有提供更多生理梯度的均匀营养和气体交换以及整体氧化的优点，并且通过碳酸氢盐缓冲系统和 CO_2 气体交换调节整个细胞室体积的 pH 值，而剪切力可忽略不计。这种生物反应器为细胞类型的持续长期培养提供了更好的环境，而不需要机械刺激。利用动态 3D 灌注生物反应器对几个细胞进行了扩增和分化研究。长期培养后，HADSCs 呈单侧空泡状脂质充盈，FABP4、GLUT4 和 PPAR-γ 阳性[45]。

4. 磁悬浮技术

另一种能够实现无支架生物制造的新技术是磁悬浮和随后的单细胞或球体的组装[20] [46]。磁悬浮可以基于正磁泳或负磁泳原理应用于细胞[47]。在正磁电泳中，用磁性纳米颗粒标记的细胞可以利用外力悬浮，这种操作可以使细胞形成无支架的 3D 结构。以前的研究表明，该技术用于 3T3-L1 前脂肪细胞和 bEND.3 内皮细胞的共培养和单培养小鼠 SVFs 的成脂潜力。免疫荧光图像显示，生成组织的脂肪生成和血管生成与天然脂肪组织相似[48] [49]。尽管正磁电泳磁悬浮是一种无接触且简单的方法，但它需要额外的标记步骤和磁性纳米颗粒(MNP)的均匀分布。不幸的是，MNP 不能从组装的生物结构中移除，并且一旦被吸收就引起细胞毒性和 DNA 损伤[50] [51] [52]。

实现磁悬浮的另一种变体是使用负磁泳，它可以根据细胞和生物体避开强磁场的趋势，使它们悬浮[53] [54] [55]。由于其无需标记 MNP 的工作原理，这是无接触负磁电泳一个额外的优势[56]。负磁泳磁悬浮可用于非生物物体和细胞的 3D 自组装[46] [57] [58] [59]。在以前的研究中，磁悬浮被用作软骨和癌细胞球体的无支架生物制造[60] [61]，以及干细胞、癌细胞和成纤维细胞的单相或双相组装[20] [62]。基于负磁电泳的磁悬浮根据细胞固有的单细胞密度分布对细胞进行分层[54] [63]，所有以前的研究都表明使用类似单细胞密度的细胞进行生物制造[20] [64]。然而，脂肪组织包含具有高度可变单细胞密度表型的细胞。以前，脂肪组织的无支架 3D 生物制造是通过磁悬浮和使用氧化铁和金纳米粒子的正磁电泳进行[48] [65]。Sarigil 利用微管装置，在负磁泳悬浮培养过程中，发现低密度的脂肪细胞导致附着在微管通道的顶部表面。因此通过降低乳浓度或与密度较高的细胞共培养可以克服这一问题。共聚焦显微镜图像显示，干细胞形成疏松结构，生长细胞紧密包裹[66]。

5. 总结

自组装、生物反应器以及磁悬浮技术的无支架脂肪组织工程虽然在实验方面取得巨大进展，然而，在临床上直接比较支架技术和无支架技术的研究很少。直接比较这两种方法仍然至关重要，特别是考虑到基于支架组织工程临床成功。未来的研究应致力于无支架组织工程向临床实践应用的转化。因此，无支架技术所涉及的异种或异体材料或合成材料的安全性是一个需要进一步考虑的问题。

总的来说，无支架 3D 细胞培养技术已经成为构建人工脂肪组织的重要手段。该技术可以用于脂肪细胞、脂肪干细胞和其他类型的细胞的培养，可以构建出具有生物完整结构和功能的人工脂肪组织。该技术的发展已经推动了脂肪组织工程领域的进步，为临床治疗和研究提供了更好的工具和平台。然而，这一技术的应用还面临一些挑战和限制，包括生长因子和细胞密度的控制、材料的选择和性质的优化、技术的可重复性和规模化等。在生长因子和细胞密度的控制方面，现有的无支架 3D 细胞培养技术尚不能完全模拟自然环境中的生长条件。因此，研究人员需要进一步研究和优化技术，以提高细胞的存活率、增殖率和分化效率。同时，材料的选择和性质的优化也是该技术应用中的关键问题。由于构建人工脂肪组织需要使用多种材料，如水凝胶、生物纤维素和聚合物等，因此研究人员需要选择合适的材料，并进行结构和性质的调节和优化，以提高材料的生物相容性和机械性能。此外，技术的可重复性和规模化也是该技术应用中的重要挑战。虽然目前的无支架 3D 细胞培养技术已经取得了一定的成功，但其应用仍受限于技术的可重复性和规模化。因此，研究人员需要进一步优化技术流程和标准化操作流程。尽管在应用中还存在一些挑战和限制，但无支架 3D 细胞培养技术已经取得了显著的进展，为构建具有完整结构和功能的人工脂肪组织提供了可能性。未来，我们可以期待该技术的不断发展和优化，为临床治疗和研究带来更多的机会和挑战。

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