·综 述·

脱细胞基质生物墨水制备方法及应用进展

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摘 要: 组织器官脱细胞后制备成的脱细胞基质 (Decellularized extracellular matrix，dECM) 含有许多蛋白质和生长因子，不仅能够为细胞提供三维支架还能够调控细胞再生，是目前最具有生物结构的生物材料。3D 生物打印可以层层打印 dECM 和自体细胞的组合，构建载细胞组织结构。文中综述了不同来源的组织器官脱细胞基质生物墨水制备方法，包括脱细胞、交联等，以及脱细胞基质生物墨水在生物打印中的应用，并展望了其未来的应用前景。

关键词: 3D 生物打印，组织工程，生物墨水，脱细胞基质

Preparation and application of decellularized extracellular matrix bioink: a review

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Abstract: Decellularized extracellular matrix (dECM), which contains many proteins and growth factors, can provide three-dimensional scaffolds for cells and regulate cell regeneration. 3D bioprinting can print the combination of dECM and autologous cells layer by layer to construct the tissue structure of carrier cells. In this paper, the preparation methods of tissue and organ dECM bioink from different sources, including decellularization, crosslinking, and the application of dECM bioink in bioprinting are reviewed, with future applications prospected.

Keywords: 3D bioprinting, tissue engineering, bioink, decellularized extracellular matrix

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自 2003 年首次报道载细胞打印证实了打印器官的可能性后[1]，3D 生物打印技术常用于生物医学中生物支架的构建上[2]。其中，生物墨水是制约 3D 生物打印效果的关键因素之一。生物墨水即在生物打印制造中能够包含细胞和其他生物活性成分的材料[3]，是目前的研究热点。近年来，研究者相继开发出了陶瓷、聚合物、弹性体和脂类等生物墨水，并取得了一定的应用进展。

脱细胞基质[4] (Decellularized extracellular matrix，dECM) 作为天然聚合物的一种，具有含细胞生长和分化所需因子以及各种蛋白质[5]、可以调节生物平衡，以及较低的毒性和免疫原性[6]等优点，在组织平衡、生长和成熟中起着至关重要的作用。并且相较于其他天然聚合物如海藻酸钠[7]、胶原蛋白[8]、琼脂糖[9]和透明质酸[10]等更具有仿生性，还能通过一定方法形成具有三维网络结构的水凝胶，提供可容纳大量水并且细胞友好的环境，因此，可以在体外模拟细胞体内生长的微环境[11-12]。

dECM 不仅在结构和成分上与天然细胞外基质相似并能促进组织再生，还可诱导宿主体内干细胞增殖分化，调控细胞信号通路和基因表达，符合生物墨水需要满足的可印刷性、良好的机械性能以及生物相容性[13]等特点。因此，dECM 生物墨水和生物打印结合的打印方案不仅可以解决组织工程上不能获得理想支架的问题，如无法对支架孔隙孔径等进行精确控制[14]、2D 细胞培养中与体内环境不符的缺陷[15]等，还能进行器官和组织制造的临床应用[16]，现有的研究表明已开发多种器官的 dECM 生物墨水并合理应用[17]，例如肺[18]、肾[19]、脑[20]、脊髓[21]、骨[22]等，可用于制造组织结构、药物筛选以及靶向药物和/或细胞递送。本文针对不同来源的 dECM 的制备及其应用进行综述，能够较全面地了解 dECM 生物墨水的前期制备，主要包括脱细胞和交联，以及后续可行性及相关应用，为今后 dECM 作为生物墨水的开发及进一步实现组织工程应用加工提供参考。

1 dECM 生物墨水的制备方法

1.1 组织器官的来源

一般来讲，dECM 的主要来源有动物源组织器官、植物组织以及人源组织器官。其中，由于人源组织器官面临伦理、法律等问题，其研究应用较少，近年来，分别在上肢[23]、卵巢[24]、皮肤[25]以及肺[26]等方面有一定探讨。

目前，动物源组织器官因其易获取、丰富且可持续，能解决组织器官的短缺问题[27]，已广泛应用于 dECM 的制备。例如，猪[28-29]、牛[30]、山羊[31]和大鼠[32]的组织器官，被用作制备 dECM 生物墨水，具有良好的细胞相容性。但动物源 dECM 在植入体内后可能引起免疫排斥反应，还有待进一步深入研究[33]。

近年来，有研究表明植物源组织因其交互的网络结构、较大的表面积、不同程度的亲水性以及较好的机械性能使其成为组织工程中血管化的合适候选材料[34]。例如，欧芹茎具有较高的亲水性和较大的孔径，可以改善细胞的粘附和增殖，并且细胞培养显示细胞沿着植物微观结构排列，揭示了结构形状在未来研究中的重要性[35]。然而植物源 dECM 的机械性能无法支撑其植入体内，需要进一步完善其制备过程。

1.2 组织器官脱细胞方法

脱细胞是指在保护天然细胞外基质的组成和结构完整性的同时，有效地去除组织或器官中的 DNA 和 RNA 等成分，从而达到避免异种移植物和同种异体移植物易因细胞抗原引起排斥反应的目的[27]。常用的脱细胞方法有物理法 (冻融法、机械搅拌法、压力法、超临界流体法等)[36-37]、化学法 (处理试剂包括酸、碱、非离子型除垢剂、离子型除垢剂、两性离子除垢剂、低渗和高渗盐水、醇类、磷酸三丁酯等)[38-39]和生物法 (酶、螯合剂和毒素等)[40]等 (表 1)。这些方法单独使用时

均会破坏 ECM 的成分、力学特性及植入体内后的宿主反应，需要进一步优化脱细胞方法[41-43]。

近年来，为了减少传统脱细胞方法对 dECM 造成的不利影响，在对组织器官进行脱细胞处理时更偏向于以上脱细胞方法的联合应用[44-45]。胡东等[46]联合冻融和灌注法对大鼠肾脱细胞，得到了更为理想且有效的大鼠肾脱细胞支架，为肾脏组织工程构建再生肾脏以及肾脏体外研究奠定了一定基础。Roth 等[47]通过冻融循环和非离子除垢剂 Triton X-100 的联合处理对肌腱进行脱细胞处理。同时，Tavassoli 等[48]采用物理和化学相结合的方法对肌腱进行脱细胞。结果表明，在液氮中快速冻融 5 个循环，加上 2.5%十二烷基硫酸钠处理 4 h，脱细胞效果最佳。Lee 等[49]用 0.5% SDS 和 0.1% NH_4OH 成功制备了骨 dECM，弥补了硬组织脱细胞方法研究的不足。

虽然联合脱细胞方法相比传统方法单独使用时对细胞外基质损伤更小，但现有的脱细胞方法对细胞外基质有着不可避免的不良影响，需要在后续制备过程中进一步改善 dECM 力学、结构和生物学性能，以弥补脱细胞过程中细胞外基质的损伤。

1.3 dECM 在生物打印中形成交联结构的方法

生物墨水在打印过程中形成稳定的交联结构有利于提升 3D 生物打印结构的机械性能，并提供细胞友好型环境。目前，常用的交联方法包括添加化学交联剂[50-51]、天然交联剂[52-53]进行交联以及物理法[54] (表 2)。其中，物理交联方法主要有光氧化法[55]和热脱氢法[56]。因它们不易控制交联条件，目前单独应用的研究相对较少。

表 1 不同脱细胞方法的应用

Table 1 Application of different decellularization methods

Methods	Sources	Decellularize process	References
Chemical method	Rat kidney	1. 1% triton X-100, 70 mL/h, 90 min 2. PBS, 30 min 3. 1% SDS, 90 min 4. PBS, 30 min 5. 0.9% NaCl, 60 mL/h, 60 min 6. Penicillin+streptomycin	[38]
	Pig kidney	1. PBS, 25 mL/min, 30 min 2. 0.1% SDS until the color turns white 3. PBS, 8 h 4. 0.1% CH_3COOOH, 2 h 5. PBS, 12 h	[39]
Chemical+physical+ enzyme method	Human kidney	1. Stored at −80 °C 2. 2×PBS+penicillin+streptococcin 15min 3. 0.02% trypsin, 1 h; 2% tween-20, 2 h; 4% sodium deoxycholate, 3 h; 1% SDS overnight 4. 2×PBS+penicillin+stallimycin, 4 h+1×PBS, 4 h	[36]
Enzyme method	Rat sciatic nerve	1. Ultra pure water, 1 h 2. 0.5% triton X-100, 48 h 3. Ultra pure water, 48 h 4. DNase, RNase, 37 °C 12 h	[40]
Chemical+physical method	Rat heart	Freeze drying at −80 °C 1. 0.02% trypsin, 0.05% EDTA, 0.05% NaN_3, 20 min 2. 1% SDS, 0.05% NaN_3, 10 min 3. 3% triton X-100, 0.05% EDTA 0.05% NaN_3, 10 min 4. 4% DCA, 5–10 min	[44]

表 2 不同交联剂在 dECM 生物支架中的应用

Table 2 Application of different crosslinking agents in dECM scaffolds

Methods	Crosslinking agents	Effects	Advantages/disadvantages	References
Chemical crosslinker	Glutaraldehyde	Glutaraldehyde reacts with amino groups in protein molecules to improve the strength, hardness and anti degradation performance of materials	Toxic, easy to cause tissue calcification	[42]
	Carbodiimide	Carbodiimide is crosslinked with collagen to improve the properties	Low toxicity; no calcification inhibition	[43]
Natural crosslinker	Genipin (GP)	Genipin reacts with free amino groups of lysine, hydroxylysine and arginine in some biomaterials	Low toxicity; stable and robust enzyme resistance	[52]
	Procyanidins (PC)	Proanthocyanidins form hydrogen bonds with elastin and elastin in collagen	Non-toxic; it has antioxidant, anti-inflammatory, anti-calcification and cardiovascular protective effects	[53]

通过化学交联剂交联，可以提高组织的力学性能，但大部分化学交联剂如环氧化合物、碳二亚胺 (CDI)、戊二醛 (GA)、己二胺氨基甲酸酯 (HMDC) 等具有毒性，此外，还可能导致体内严重的免疫排斥反应和钙化等不良结果[53,57-58]。

近年来，天然交联剂[44-45,59]因其含有较低的细胞毒性并且对 dECM 的组成改变较小，受到广泛关注。Wang 等[60]对比使用化学交联剂戊二醛和天然交联剂京尼平来降低猪肝 dECM 的免疫原性，结果表明与化学交联方法相比，使用京尼平交联降低了 dECM 异种移植后的免疫反应，表明京尼平具有很好交联 dECM 的潜力。在另一项研究中，Wang 等[53]报道了原花青素可以交联弹性蛋白并抑制其引发的钙化，同时保持了天然结构，具有大孔径以及高孔隙率。说明 PC 交联 dECM 可促进体内再细胞化，并显示出有效的抗血栓、血液相容性和抗炎潜力。但基于天然交联剂的交联方法价格昂贵且需要较长的凝胶时间，这并不适合用于结构复杂的组织，例如 3D 肿瘤模型[61]。

2 dECM 在 3D 生物打印中的应用进展

dECM 生物墨水具有类似细胞生长增殖所需的生长环境，已被广泛应用于组织工程的研究。目前已有研究将 dECM 结合细胞应用于生物打印中进行特异性组织的构建，为今后 dECM 临床应用奠定重要基础。

2.1 中枢神经系统的修复

dECM 本身具有一定的消炎作用，对于中枢神经系统的构建和修复，可以起到消除障碍的作用。Hong 等[62]制备的猪脑 dECM 和Ⅰ型胶原蛋白复合水凝胶，通过影响巨噬细胞的活动来进行消炎，达到治疗脊髓损伤小鼠的目的，证明 dECM 可以解决哺乳动物中枢神经系统修复障碍的问题。但是凝胶降解速度过快会使消炎作用未来得及发挥就降解，这也是限制其应用的一个因素。

Tukmachev 等[63]、Medberry 等[21]以及 Crapo 等[64]分别制备基于猪脊髓 dECM、猪脑 dECM 以及基于猪脊髓 dECM 的水凝胶，发现中枢神经系统衍生的脱细胞基质能够刺激新生血管形成和轴突向内生长，修复脊髓损伤。但是只有脑 dECM 增加了轴突的长度，表明存在组织特异性效应。神经系统水凝胶对三维轴突延伸的支持也表明，这些水凝胶提供了促进体内轴突修复所需的支架。在中枢神经系统再生医学应用中提供组织特异性优势。

2.2 类肾脏组织结构的构建及应用

在组织工程中，肾脏的供体短缺一直是一个问题，亟需探索肾脏组织结构的构建问题。Ali等[54]以猪肾 dECM 为生物墨水的一部分，混合明胶、透明质酸、甘油制成复合生物墨水，提供了所需的印刷性和结构完整性，以及肾特异性微环境，可以支持人肾细胞成熟和组织形成。另外，由于肾脏是一个高度血管化的器官，所以现有的研究主要以开发肾组织类似物为主，用于肾毒性预测、药物筛选和疾病建模[65]。Singh 等[66]制备了猪肾 dECM、海藻酸钠复合生物墨水，通过数字可调协同轴打印技术，根据所需要的间隔切换沿管长度，打印出单层、双层结构和复杂可灌注空心管结构。这是细胞微流控/血管化肾小管组织的打印肾复杂结构的基础。

2.3 类食道组织结构的构建及应用

常规的食道疾病治疗手段是用大肠、皮肤组织等来修复食管组织，手术过程及术后会引起术后并发症和外科疾病。因此，3D 生物打印食道成为最优选择。Nam 等[67]开发了一种新的拖动打印方式,能够控制单个挤压过程中孔隙的大小和形状。通过对打印聚己内脂 (Polycaprolactone，PCL) 过程孔隙和孔径进行控制，打印出具有食道特性的 PCL 支架后，向不同层之间注入载有食管组织黏膜和肌肉层细胞的猪食道 dECM 溶液，形成具有多层特征的三维食管结构，应用于食管组织再生。

2.4 类肝脏组织结构的构建及应用

肝脏是人体的重要器官，能够为体内代谢物或化学物质解毒[68]。通常通过肝移植来治疗肝脏疾病，可是肝脏的需求远远大于供给。Lee 等[69]使用 PCL 与猪肝 dECM 混合制成复合生物墨水，干细胞分化和细胞功能在猪肝 dECM 生物墨水中表现良好，表明猪肝 dECM 能诱导干细胞分化，增强 HepG2 的细胞功能。Mao 等[70]将甲基丙烯酸明胶 (GelMA) 与肝 dECM 相结合，通过光固化制备了肝 dECM 特异性复合生物墨水，并将人诱导的肝细胞 (hiHep 细胞) 包裹成载细胞生物墨水，打印出具有内齿轮状结构的肝脏微组织有利于肝脏功能的恢复。这些研究中肝脏 dECM 生物墨水具有良好的可印刷性，且打印过程中没有明显的细胞死亡，进一步证明了肝脏 dECM 可以应用到肝脏的组织工程中。

2.5 类肺组织结构的构建及应用

将肺 dECM 应用到组织工程上可以为患有肺部疾病的患者提供一种有前途的解决方法。Gupta 等[31]对山羊肺 dECM 进行了探究，证实了山羊肺 dECM 具有抗菌性以及良好的生物相容性。研究进一步证明了肺 dECM 应用到肺组织工程支架的潜在可行性。Young 等[71]为探究肺 dECM 是否必须通过丢失蛋白来增强，以实现适当的上皮屏障形成,对 dECM 涂层的上皮屏障功能进行了评估，通过比较不同组合的Ⅰ型胶原、纤维连接蛋白、层粘连蛋白和 dECM 作为体外涂层的屏障功能，证明具有层粘连蛋白的 dECM 肺泡上皮具有更好的屏障功能。

2.6 类心脏组织的构建及应用

Jang 等[72]首次使用维生素 B2 作为光引发剂，利用 UVA 辐照进行交联猪源心脏 dECM。维生素 B2 和 UVA 辐照交联 dECM 的结构比热交联 dECM 结构硬度高 33 倍。Yu 等[73]也利用基于数字光处理 (Digital light processing，DLP) 的可光交联的组织特异性猪源心脏 dECM 生物墨水，制造具有高度控制复杂微结构和机械性能的患者特定组织 (图 1A)。另外，只有从心脏左心室获得的心脏 dECM 水凝胶才能进入治疗心肌梗死的临床试验。Traverse 等[74]将猪心 dECM 和纳米黏土混合制成一种注射生物墨水替代传统栓塞剂用于经导管栓塞手术，实验证明，dECM 水凝胶具有促生、抗菌性能和良好的机械特性，以及在

抗凝血液中的优异性能，与纳米黏土形成的复合水凝胶能够发挥更好的作用。

2.7 类脂肪组织的构建及应用

脂肪的形成是由组织微环境中的因素调节的，包括生长因子、激素和细胞外基质。dECM的机械性能以及生化特性会影响脂肪细胞功能[75]。Uriel 等[76]制备脂肪 dECM 生物墨水，表明了可以在体外提供支持脂肪细胞聚集和分化的环境，以及在体内血管化脂肪的形成。Choi 及其同事[77]证明猪脂肪 dECM 通过诱导脂肪生成可作为异种移植组织工程的替代材料。此外，Pati 等[78]平行制备了猪脂肪 dECM (adECM) 等 3 种不同器官来源的 dECM 水凝胶，在研究 adECM 过程中的高密度条件 (5×10^6 个细胞/mL)下，脂肪分化增强，细胞形态更圆，小鼠体内实验可看出有血管生成且体内存在动态重塑 (图 1B)。

2.8 类皮肤组织的构建及应用

皮肤 dECM 中含有层粘连蛋白 b3、胶原Ⅳ和胶原Ⅶ，这些都是皮肤具有一定功能所必需的。Pilipchuk[79]用戊二醛交联大鼠真皮 dECM，并对制成的水凝胶进行测试，后发现经过戊二醛处理的水凝胶能够增强力学特性、缓解降解和延长使用寿命。Ahn 等[80]使用猪皮来源的 dECM 作为生物墨水进行打印制备生物支架，并通过修改原本的打印机形成了一种新的打印系统，达到每层生物墨水凝胶的同时精确生成细胞负载结构，并且不影响打印后细胞的存活能力，具有高保真的三维结构以及高细胞活力。这些材料在体外和体内诱导血管化组织形成，表明它们在组织工程中具有重要的潜力。

2.9 类血管组织的构建及应用

3D 同轴生物打印因为能够通过耦合的核、壳喷嘴打印生物材料，显示了血管组织工程的潜力，直接制造可灌注的血管模拟结构进行应用。Gao 等[81]同轴打印了含有血管 dECM 和海藻酸钠的复合生物墨水，制得生物血管(BBV)。该血管可以将内皮细胞和促血管生成药物 (阿托伐他汀) 输送到缺血损伤部位，并且促进细胞的增殖、分化和新生血管的生成 (图 1C)。另外，有些研究考虑到成本以及批量差异问题，所以运用植物 dECM 避免这一问题。植物组织含有硬成分和软成分[82]，排列在复杂的层次结构中，有利于其在组织工程支架上的应用。Hickey 等[83]用苹果 dECM 皮下植入小鼠背部，证明存在血管生成和细胞迁移 (图 1D)。

2.10 类软骨组织的构建及应用

软骨是一种无血管承载组织，其低细胞性和无血管特性导致软骨损伤后自我修复能力有限[84]，应用移植物可能影响修复结果[85]。Rothrauff 等[86]通过从关节软骨和半月板中提取软骨 dECM 在载细胞打印后发现该生物墨水在促进骨髓基质细胞增殖方面发挥了重要作用。Luo 等[87]将另一种成体干细胞接种在软骨 dECM 支架上，也观察到细胞增殖增强。这些研究表明软骨 dECM 为软骨细胞黏附、增殖提供一个更合适的三维环境。

2.11 类骨组织的构建及应用

目前常用打印骨支架的生物墨水有生物陶瓷、水凝胶、非水凝胶聚合物及其复合材料。Lee 等[88]制备了甲基丙烯酸脱细胞基质 (Ma-dECM) 与海藻酸钠复合生物墨水，相比于海藻酸钠具有更高的细胞存活率，并且骨 dECM 可诱导更高的细胞活性。Choi 等[89]通过将静电纺丝制备的 PCL 纤维支架包裹骨脱细胞基质制备生物支架。相比 PCL，骨 dECM 对支架力学特性没有影响，但是对细胞黏附和增殖以及分化有显著影响。因此，运用骨脱细胞基质复合材料生物墨水不仅可以使支架具有骨传导性，还改变支架的物理特性和生物学性能，进一步满足骨组织工程的需求。

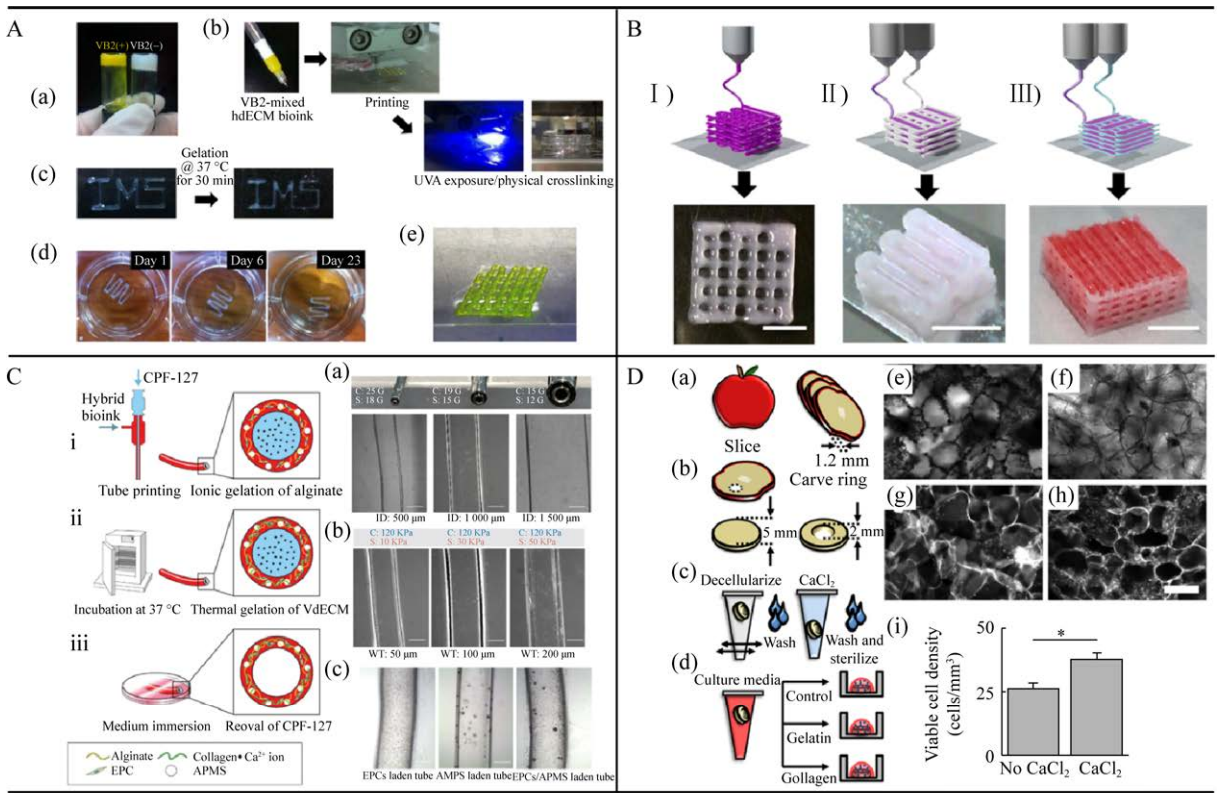

图 1　基于脱细胞基质的生物墨水的应用[83]

Fig. 1　Application of bioink based on dECM. (A) a: gelation behavior of heart dECM (hdECM) bioink with (+) or without (–) vitamin B2 (VB2). b: procedure to induce covalent crosslinking of dECM by exposure to UVA light during printing, followed by physical crosslinking of dECM bioink after printing. c: printed ‘‘IMS”-shaped structure and physical stability after two-step crosslinking. d: structural stability of printed and two-step crosslinked structure for 23 days in normal saline. e: an image of a 10 layer printed bioconstruct[72]. (B) Ⅰ: heart tissue construct was printed with hdECM. Ⅱ: cartilage and adipose tissues were printed with cartilage dECM (cdECM) and Ⅲ adipose dECM (adECM) respectively, and in combination with PCL framework[79]. (C) Fabrication and structural configuration of the BBVs. i: the ionic gelation of alginate in realized BBV printing, ii: the thermal crosslinking of collagen fibers was induced by incubation at 37 °C, iii: medium immersion dissolved and removed CPF127 to obtain BBV with a hollow tubular shape. a: combinations of various core and shell nozzles allowed the production of tubes with different inner diameters. b: adjusting the bioink flow in the shell nozzle allows BBV with different wall thickness to be achieved. c: BBV was successfully prepared[81]. (D) a–d: schematic of biomaterial preparation; e–i: a $CaCl_2$ pretreatment was used to remove remnant surfactant from the scaffold[83].

3　总结与展望

脱细胞基质是一个复杂的结构，包括传导化学信号所必需的各种蛋白及糖胺聚糖，从而影响细胞行为、组织再生、血运重建和调节内稳态。现有的研究表明，dECM 由于其自身的生物学优势，在组织工程领域具有广阔的应用前景并得到了发展，在很多方面，如组织工程、细胞移植、体内外建模以及药物传递中都显示出了巨大的优势和潜力。

尽管已有很多研究表明 dECM 材料可以应用于临床治疗中，但 dECM 在应用中仍面临着许多难题，未来可以从以下几个方面深入研究：(1) 优化 dECM 生物墨水制备方案。脱细胞过程中残留的 DNA 可能产生免疫反应，且容易导致血栓的形成和炎症。因此需要制定标准化的脱细胞方案。

在去除核酸的同时更完整地保留其他活性成分和结构；另外，寻找安全有效的交联方法，在保证安全性的前提下改善生物墨水的机械性能，可以扩大生物墨水的应用范围。(2) 细胞外基质与细胞和体内微环境相互作用的更详细机制目前尚不清楚。除此之外，需要控制脱细胞基质的可降解性。降解后的 dECM 可释放内皮生长因子、转化生长因子等，可局部促进血管生成以及诱导细胞向损伤部位迁移修复。但是脱细胞基质促进细胞行为、组织再生和血管生成的具体组成尚不清楚，相关的细胞和分子机制也值得研究。(3) 现有的研究都是在实验室中进行的，扩大生产规模的同时提高 dECM 批次的可重复性的转变也是一项挑战。

随着以上难题的解决，具有更好的生物相容性和功能重建的 dECM 生物墨水将不断涌现。在此之前，还需要不断加深对其作用机制的认识，不断改进技术，采用更多的方法，结合更广泛的知识背景，使其具有更广泛的应用。

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