

[DOI]10.12016/j.issn.2096-1456.2023.01.004

· 基础研究 ·

3D打印白硅钙石支架材料在大鼠 Onlay 骨移植中的应用

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【摘要】 目的 评价3D打印白硅钙石(bredigite, BRT)骨支架材料在大鼠 Onlay 骨移植中的效果。方法 通过3D打印技术制备骨BRT支架材料作为实验组, β -磷酸三钙(β -tricalcium phosphate, β -TCP)骨支架材料作为对照组, 通过扫描电镜(scanning electron microscopy, SEM)、X射线衍射(X-ray diffraction, XRD)、力学测试和体外降解测试实验对其进行表征观察。建立SD大鼠颅骨 Onlay 骨移植的动物模型, 根据移植物的不同, 分为自体骨移植组(Auto组)、 β -TCP组及BRT组。术后12周获取标本行大体观察、micro-CT扫描检测分析、组织学HE染色。**结果** SEM观察显示, BRT有规则的多孔结构, XRD衍射峰较为锐利。与 β -TCP组(11.29 ± 1.30) MPa相比, BRT支架材料(46.80 ± 3.44) MPa具有更好的力学强度($P < 0.001$); 在体外第35天时, BRT降解率为 $27.18\% \pm 1.41\%$, 并且在降解过程中释放钙、镁、硅离子。micro-CT及组织学染色实验结果提示, Auto组有一定程度的骨吸收, β -TCP和BRT组均有一定新骨生成。micro-CT和组织学定量结果, BRT组新生骨组织占比分别为 $16.83\% \pm 2.11\%$ 和 $19.08\% \pm 2.17\%$, 均高于 β -TCP组($8.48\% \pm 1.85\%$, $10.81\% \pm 1.33\%$) ($P < 0.05$)。**结论** 3D打印BRT骨支架材料促进 Onlay 骨移植中的骨再生。

【关键词】 Onlay 骨移植; 白硅钙石; β -磷酸三钙; 自体骨; 骨量不足; 种植; 3D打印; 骨支架材料; 骨组织工程; 动物模型; 骨再生

【中图分类号】 R78 **【文献标志码】** A **【文章编号】** 2096-1456(2023)01-0017-06

【引用著录格式】 章臻, 李佳洋. 3D打印白硅钙石支架材料在大鼠 Onlay 骨移植中的应用[J]. 口腔疾病防治, 2023, 31(1): 17-22. doi:10.12016/j.issn.2096-1456.2023.01.004.

Application of 3D-printed bredigite scaffolds in Onlay graft in rats ZHANG Zhen¹, LI Jiayang². 1. Department of Oral & Maxillofacial-Head & Neck Oncology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine; College of Stomatology, Shanghai Jiao Tong University & National Center for Stomatology & National Clinical Research Center for Oral Diseases & Shanghai Key Laboratory of Stomatology, Shanghai 200011, China; 2. Shanghai Stomatological Hospital, Fudan University; Shanghai Key Laboratory of Craniomaxillofacial Development and Diseases, Fudan University, Shanghai 200001, China

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【Abstract】 Objective To evaluate the osteogenic effects of using a 3D-printed bredigite(BRT) bone scaffold on the Onlay graft in rats. **Methods** BRT scaffold material was fabricated by 3D printing technology as the experimental group, β -tricalcium phosphate(β -TCP) bone scaffolds were used as the control group. The scaffolds were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), mechanical test and in vitro degradation test. An animal model of Onlay graft in SD rats was established. According to the different grafts, the animal model was divided into autogenous bone transplantation group (Auto Group), β -TCP group and BRT group. At 12 weeks after the operation, the target line was obtained for gross observation, micro CT scanning and analysis, and HE staining histological analysis.

【收稿日期】 2022-07-25; **【修回日期】** 2022-08-08

【基金项目】 国家自然科学基金项目(81900968); 上海市青年科技英才扬帆计划项目(19YF1426100)

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Results BRT scaffolds have distinctive surface topography with relatively uniformly sized pores in SEM imaging. A higher mechanical strength and degradation rate was obtained with BRT scaffolds (46.80 ± 3.44) MPa, compared to β -TCP scaffolds (11.29 ± 1.30) MPa ($P < 0.001$). At day 35 *in vitro*, the mass of BRT was degraded $27.18\% \pm 1.41\%$, and the Ca, Mg and Si ion concentrations were maintained at a high level in BRT group. Micro CT and histological quantitative results showed that the proportion of bone regeneration in BRT group was $16.83\% \pm 2.11\%$ and $19.08\% \pm 2.17\%$, respectively, which was higher than that in the β -TCP group ($8.48\% \pm 1.85\%$, $10.81\% \pm 1.33\%$) ($P < 0.05$).

Conclusion 3D-printed BRT scaffolds promote bone regeneration in the Onlay graft.

【Key words】 Onlay graft; bredigite; β -tricalcium phosphate; autogenous bone; bone insufficiency; implant; 3D printing; bone scaffolds; bone tissue engineering; animal model; bone regeneration

J Prev Treat Stomatol Dis, 2023, 31(1): 17-22.

【Competing interests】 The authors declare no competing interests.

This study was supported by the grants from National Natural Science Foundation (No. 81900968) and Shanghai Sailing Program (No. 19YF1426100).

缺牙区牙槽骨有足够的骨量是口腔种植修复技术的成功及远期效果的保证,而大部分种植患者伴有缺牙区骨量不足,其中重度骨量不足患者约占其中的30%^[1-2]。外置法自体骨移植(Onlay graft)依然是目前解决缺牙区重度骨量不足的有效手段,但其具有需开辟第二术区、容易发生供区并发症以及自体骨吸收率较高等缺点^[3-5]。

骨组织工程发展至今,已有大量具有良好生物相容性、骨引导、骨传导、骨诱导性能的骨支架材料被研发,组织工程骨支架材料也有望为Onlay骨移植提供一个新的思路^[6-10]。而目前骨支架材料的研究多集中于骨缺损模型^[11-14],鲜有应用于Onlay骨移植的研究。白硅钙石($\text{Ca}_7\text{MgSi}_4\text{O}_{16}$) (bredigite, BRT)是一类含有钙、硅、镁三元组分的生物活性陶瓷,具有优异的生物相容性、磷灰石矿化能力和生物活性,研究证实其在体内外均具有促进血管再生和骨再生作用,是一种良好的骨支架材料^[15-16]。本实验拟构建大鼠颅骨Onlay骨移植的动物模型,并应用3D打印技术制备BRT支架材料,评价其在Onlay骨移植中的成骨效果,以期为3D打印BRT石骨支架材料在种植Onlay骨移植的临床应用提供实验依据。

1 材料和方法

1.1 材料与仪器

白硅钙石(BRT), β -磷酸三钙(β -tricalcium phosphate, β -TCP)材料由烟台正海生物有限公司提供。SD大鼠由上海交通大学医学院动物实验中心提供,动物合格证号[SYXK(沪)2016-0016]。扫描电镜(S-4800,日立,日本);X射线衍射仪(D8

Advance, Bruker, 德国);电感耦合等离子体光学发射光谱仪(ICP-OES 720ES, Agilent, 美国);微机控制电子万能试验机(ETM204C, 北京博医实验仪器有限公司, 中国);显微CT(vivaCT80, Scanco Medical, 瑞士);光学电子显微镜(DP71, Olympus, 日本)

1.2 支架材料的制备

以正硅酸乙酯、水、六水硝酸镁以及氯化钙作为原材料,采用溶胶-凝胶法制备获取BRT粉末, β -TCP作为对照组。将BRT粉末、树脂及各种添加剂混合,制备获得打印浆料。将设计好的支架模型,转换成STL格式,导入打印机内。设置打印参数,包括扫描速度激光的功率、激光半径。将打印浆料放入料槽中,执行打印,获取支架材料。

1.3 支架材料的表征观察

1.3.1 扫描电镜(scanning electron microscopy, SEM)

观察支架材料表面的微观形态 支架材料镀金后,通过SEM在加速电压下进行检测,获得支架材料表面的微观形态图像。

1.3.2 X射线衍射(X-ray diffraction, XRD)进行物相分析 用XRD仪对材料行物相分析,扫描角度为 $10^\circ \sim 80^\circ$,扫描速率为 $5^\circ/\text{min}$ 。

1.3.3 支架材料力学测试 支架材料置于电子万能试验机上进行力学试验。室温环境下进行测试,采用1 kN的传感器,压缩速率1 mm/min。获取最大抗压强度力学数据。由公式:抗压强度=作用力/接触面积,计算相应的作用力。

1.3.4 支架材料体外降解 支架称重后浸泡在模拟体液中,分别于0、3、7、14、28、35 d取出3份,称取其质量,后计算其降解率。降解率的计算公式:降解率= $(m_0 - m_1)/m_0 \times 100\%$, m_0 为初始质量, m_1 为支

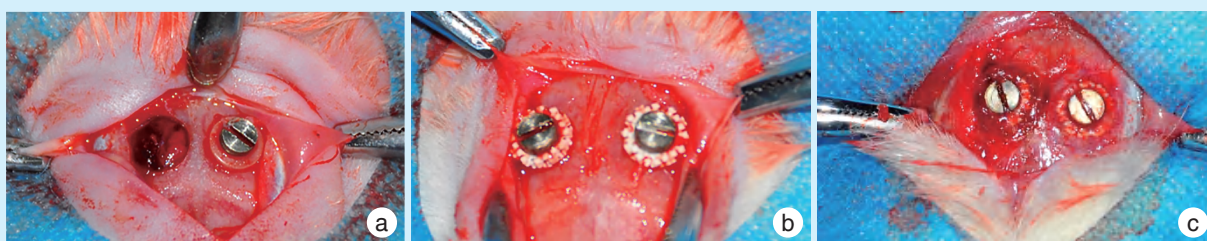
架材料降解后的质量。同时pH仪测试取出材料时溶液的pH值;电感耦合等离子体光学发射光谱仪测试溶液中钙、镁、硅离子浓度。

1.4 SD大鼠Onlay骨移植手术

本动物实验均获得上海交通大学附属第九人民医院动物伦理委员会批准(SH9H-2020-A610)。

1.4.1 实验分组 本实验使用12只SD雄性大鼠, 体质量(220 ± 18.6)g, 随机分为3组, 每组4只: 自体骨移植组(Auto组)、 β -TCP支架材料组(β -TCP组)、BRT支架材料组(BRT组)。

1.4.2 Onlay骨移植模型手术过程 动物全麻后, 常规备皮, 消毒, 铺巾。于颅骨正中皮肤做1.5 cm的矢状切口, 解剖分离暴露颅骨。在Auto组, 5 mm环钻于颅骨左侧取下圆形骨块, 受区钻孔制备受植床后, 将其用钛钉固定于右侧受植区; 在 β -TCP组和BRT组, 受区钻孔制备受植床后, 分别将 β -TCP支架材料和BRT支架材料用钛钉分别固定于颅骨左右两侧(图1)。分层对位缝合肌肉、皮肤。术后12周, 处死大鼠获取颅骨术区样本, 供后续实验备用。



a: in the autologous bone graft group, the bone block harvested on the left was grafted on the right side with a titanium screw; b: BRT scaffolds were grafted on both sides of the cranium bone with a titanium screw; c: β -TCP scaffolds were grafted on both sides of the cranium bone with a titanium screw. β -TCP: β -tricalcium phosphate; BRT: bredigite

Figure 1 Surgical procedure of Onlay graft in SD rats

图1 SD大鼠颅骨Onlay骨移植模型手术过程

1.5 micro-CT检测骨体积分数BV/TV

样本于4%的中性多聚甲醛缓冲溶液固定2 d后, 进行micro-CT扫描。扫描参数为: 70 kV、114 μ A、700 ms整合时间; 扫描区域每个像素的物理分辨率为18 μ m。断层图片行三维重建参数设定为 $\sigma=1.2$ 、support=1。设定2个阈值范围: ①60~100 HU; ②500~1 000 HU, 以此将骨组织、材料、种植钉与其它物质区分开; 感兴趣区(volume of interest, VOI)被设定为移植物周围250 μ m的环形区域, 于VOI内由micro-CT自带软件测出相对骨体积BV/TV的定量数据。

1.6 HE染色观察新生骨比例

完成micro-CT检测的样本用10% EDTA进行脱钙, 常规制备5 μ m厚的切片, 行HE染色, 正置显微镜下观察。用Image J软件进行图像分析测量切片中新生骨所占比例。

1.7 统计学分析

GraphPad Prism 8.0软件对实验数据进行统计学分析, 计量资料用 $\bar{x} \pm s$ 表示, 组间比较行独立样本t检验, $P < 0.05$ 认为差异有统计学意义。

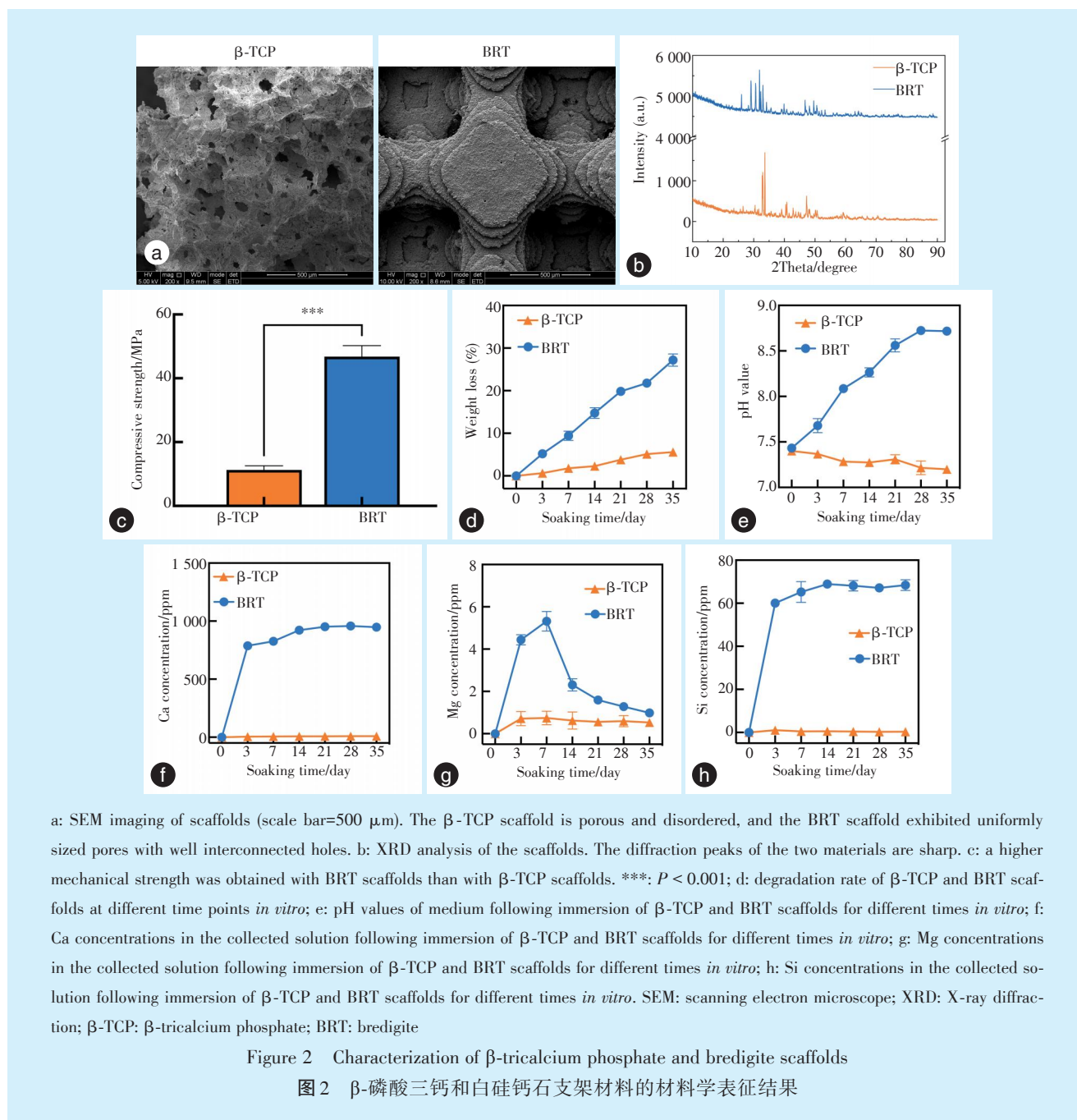
2 结果

2.1 白硅钙石支架材料的表征

支架材料的表面形貌见图2a, β -TCP和BRT支架材料均为多孔结构, BRT支架材料较为规则。XRD图谱见图2b, BRT材料衍射峰较锋利, 说明其有良好的结晶性能。力学测试结果提示, 相较于 β -TCP(11.29 ± 1.30) MPa, BRT支架材料(46.80 ± 3.44) MPa有明显更好的抗压强度($P < 0.001$) (图2c)。支架材料体外降解实验提示, BRT支架材料有更好的降解率, 在体外第35 d时, BRT降解率为 $27.18\% \pm 1.41\%$; 并且在降解过程中有钙、镁、硅离子释放(图2d~2h)。

2.2 白硅钙石支架材料应用于大鼠Onlay骨移植手术后情况

大体标本见移植物未出现松动、脱落, 自体骨、支架材料均与其下方的颅骨有一定骨融合。Micro-CT三维重建图示, 自体移植骨组边缘有轻微吸收; β -TCP组和BRT组支架材料表面有新生骨生成影像。定量分析结果提示, BRT组相对骨体积分数(BV/TV)为 $16.83\% \pm 2.11\%$, 显著高于 β -



TCP组($8.48\% \pm 1.85\%$)($P < 0.001$)(图3)。HE染色结果显示,自体移植骨出现了骨吸收; β -TCP支架材料吸收不明显,材料表面有少量新生骨;BRT支架材料有一定吸收,支架材料表面与内部有新生骨生成。定量分析结果显示,BRT组新生骨量($19.08\% \pm 2.17\%$)显著高于 β -TCP组($10.81\% \pm 1.33\%$)($P < 0.001$)(图4)。

3 讨论

本实验中建立了一个大鼠颅骨 Onlay 植骨的研究动物模型,并证实制备的3D打印BRT支架材

料在该模型中可获得一定骨再生。

目前,Onlay自体骨植骨后,自体骨的吸收不可预期是其主要缺点^[1-2],这可能导致骨移植后骨量仍不足,甚至需要再次植骨。如何减少Onlay自体骨移植的骨吸收也是口腔种植领域中的研究热点^[17-18]。临床上,用于减少Onlay自体骨吸收的手段包括:取骨区选用膜内骨成骨的颌骨(下颌升支、外斜线,下颌颈部等)^[19],受区钻孔去皮质化预备,骨块的坚固内固定^[20],无张力缝合,屏障膜覆盖等^[2,4,21]。在本实验中,BRT支架材料本身具有较好的强度可承担移植周围软组织的压力,同

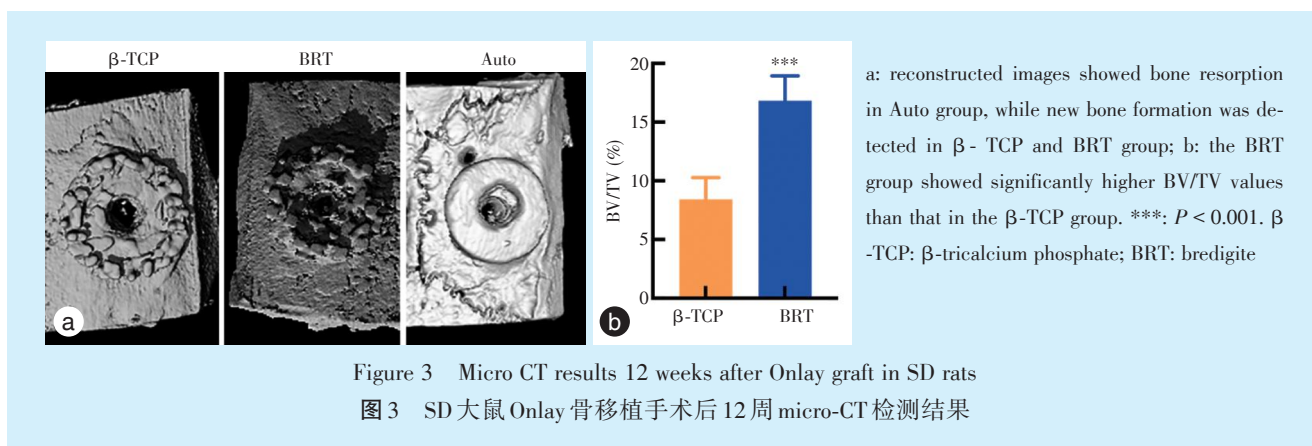


Figure 3 Micro CT results 12 weeks after Onlay graft in SD rats
图3 SD大鼠Onlay骨移植手术后12周micro-CT检测结果

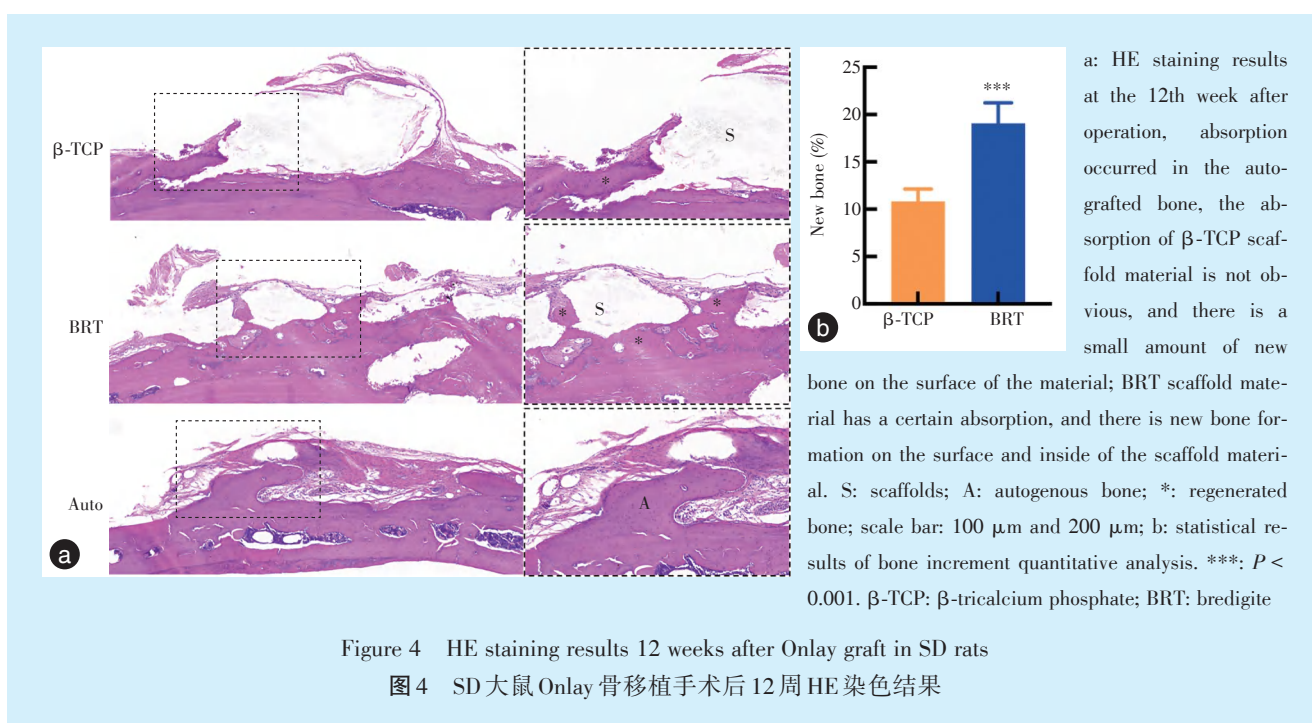


Figure 4 HE staining results 12 weeks after Onlay graft in SD rats
图4 SD大鼠Onlay骨移植手术后12周HE染色结果

时本实验同样对受植区进行了去皮质化的预备,对移植物用钛钉进行了坚固内固定保证其稳定性,也进行了无张力缝合。这些措施也保证了最后的成骨效果。

与传统的无机生物陶瓷材料羟基磷灰石、β-TCP等相比,BRT支架材料在降解过程中,释放的钙、镁、硅离子,被大量研究证实可以促进干细胞的成骨分化、成骨细胞的增殖、血管再生^[6,15,22],也解释了在本实验中,与β-TCP组相比,BRT组获得了更好的成骨效果。同时BRT支架材料降解过程中,会产生偏碱的微环境环境,相对一些高分子聚合物降解后产酸降低微环境pH值,偏碱性的微环境更适宜细胞的增殖分化,同时具有抑制细菌的作用。

临床上,种植骨量不足区域所需通常形态不

规则,常规的自体骨移植通常难以实现精准修复重建。而3D打印技术可以精准打印空间结构复杂的个性化支架材料^[23-24],实现骨支架材料宏观上满足空间形貌要求,微观上通道精密联通、孔隙结构可控^[6,7,22-23]。3D打印技术结合组织工程支架材料及数字化设计,有望在种植Onlay骨移植中被更多地应用。

综上,本实验为3D打印BRT骨支架材料应用于Onlay骨移植提供了一定依据。本实验只是单纯应用了支架材料,在后续的研究中可联用干细胞、生长因子等,并结合3D生物打印技术,进一步优化骨支架材料,有望获得更好的临床效果。

【Author contributions】 Zhang Z designed the study and wrote the article. Li JY performed the experiments and revised the article. All authors read and approved the final manuscript as submitted.

参考文献

- [1] Benic GI, Hämmerle CH. Horizontal bone augmentation by means of guided bone regeneration[J]. *Periodontol* 2000, 2014, 66(1): 13-40. doi: 10.1111/prd.12039.
- [2] Chappuis V, Cavusoglu Y, Buser D, et al. Lateral ridge augmentation using autogenous block grafts and guided bone regeneration: a 10-year prospective case series study[J]. *Clin Implant Dent Relat Res*, 2017, 19(1): 85-96. doi: 10.1111/cid.12438.
- [3] Naemi N, Lim HC, Papageorgiou SN, et al. Efficacy of lateral bone augmentation prior to implant placement: a systematic review and meta-analysis[J]. *J Clin Periodontol*, 2019, 46(Suppl 21): 287-306. doi: 10.1111/jcpe.13052.
- [4] Sáez-Alcaide LM, Brinkmann JC, Sánchez-Labrador L, et al. Effectiveness of the bone ring technique and simultaneous implant placement for vertical ridge augmentation: a systematic review[J]. *Int J Implant Dent*, 2020, 6(1): 82. doi: 10.1186/s40729-020-00280-0.
- [5] Gaikwad AM, Joshi AA, Padhye AM, et al. Autogenous bone ring for vertical bone augmentation procedure with simultaneous implant placement: a systematic review of histologic and histomorphometric outcomes in animal studies[J]. *J Prosthet Dent*, 2021, 126(5): 626-635. doi: 10.1016/j.prosdent.2020.09.001.
- [6] Khalaf AT, Wei Y, Wan J, et al. Bone tissue engineering through 3D bioprinting of bioceramic scaffolds: a review and update[J]. *Life (Basel)*, 2022, 12(6): 903. doi: 10.3390/life12060903.
- [7] Mohaghegh S, Hosseini SF, Rad MR, et al. 3D printed composite scaffolds in bone tissue engineering: a systematic review[J]. *Curr Stem Cell Res Ther*, 2021. doi: 10.2174/1574888X16666210810111754.
- [8] Ye G, Bao F, Zhang X, et al. Nanomaterial-based scaffolds for bone tissue engineering and regeneration[J]. *Nanomedicine (Lond)*, 2020, 15(20): 1995-2017. doi: 10.2217/nmm-2020-0112.
- [9] Shi R, Huang Y, Ma C, et al. Current advances for bone regeneration based on tissue engineering strategies[J]. *Front Med*, 2019, 13(2): 160-188. doi: 10.1007/s11684-018-0629-9.
- [10] Cheng A, Schwartz Z, Kahn A, et al. Advances in porous scaffold design for bone and cartilage tissue engineering and regeneration [J]. *Tissue Eng Part B Rev*, 2019, 25(1): 14-29. doi: 10.1089/ten.TEB.2018.0119.
- [11] Zhang Y, Wang C, Fu L, et al. Fabrication and application of novel porous scaffold in situ-loaded graphene oxide and osteogenic peptide by cryogenic 3D printing for repairing critical-sized bone defect[J]. *Molecules*, 2019, 24(9): 1669. doi: 10.3390/molecules24091669.
- [12] Ren J, Kohli N, Sharma V, et al. Poly- ϵ -caprolactone/fibrin-alginate scaffold: a new pro-angiogenic composite biomaterial for the treatment of bone defects[J]. *Polymers (Basel)*, 2021, 13(19): 3399. doi: 10.3390/polym13193399.
- [13] Thieu M, Haugen HJ, Sanz-Esporrin J, et al. Guided bone regeneration of chronic non-contained bone defects using a volume stable porous block TiO₂ scaffold: an experimental *in vivo* study[J]. *Clin Oral Implants Res*, 2021, 32(3): 369-381. doi: 10.1111/clr.13708.
- [14] Wang M, Yang Y, Chi G, et al. A 3D printed Ga containing scaffold with both anti-infection and bone homeostasis-regulating properties for the treatment of infected bone defects[J]. *J Mater Chem B*, 2021, 9(23): 4735-4745. doi: 10.1039/d1tb00387a.
- [15] Zhang W, Feng C, Yang G, et al. 3D-printed scaffolds with synergistic effect of hollow-pipe structure and bioactive ions for vascularized bone regeneration[J]. *Biomaterials*, 2017, 135: 85-95. doi: 10.1016/j.biomaterials.2017.05.005.
- [16] Shao H, Sun M, Zhang F, et al. Custom repair of mandibular bone defects with 3D printed bioceramic scaffolds[J]. *J Dent Res*, 2018, 97(1): 68-76. doi: 10.1177/0022034517734846.
- [17] Dam VV, Trinh HA, Rokaya D, et al. Bone augmentation for implant placement: recent advances[J]. *Int J Dent*, 2022, 2022: 8900940. doi: 10.1155/2022/8900940.
- [18] Farias D, Caceres F, Sanz A, et al. Horizontal bone augmentation in the posterior atrophic mandible and dental implant stability using the tenting screw technique[J]. *Int J Periodontics Restorative Dent*, 2021, 41(4): e147-e155. doi: 10.11607/prd.5137.
- [19] Severi M, Simonelli A, Farina R, et al. Effect of lateral bone augmentation procedures in correcting peri-implant bone dehiscence and fenestration defects: a systematic review and network meta-analysis[J]. *Clin Implant Dent Relat Res*, 2022, 24(2): 251-264. doi: 10.1111/cid.13078.
- [20] Thoma DS, Bienz SP, Figuero E, et al. Efficacy of lateral bone augmentation performed simultaneously with dental implant placement: a systematic review and meta-analysis[J]. *J Clin Periodontol*, 2019, 46(Suppl 21): 257-276. doi: 10.1111/jcpe.13050.
- [21] Von Arx T, Buser D. Horizontal ridge augmentation using autogenous block grafts and the guided bone regeneration technique with collagen membranes: a clinical study with 42 patients[J]. *Clin Oral Implants Res*, 2006, 17(4): 359-366. doi: 10.1111/j.1600-0501.2005.01234.x.
- [22] Chen L, Deng C, Li J, et al. 3D printing of a lithium-calcium-silicate crystal bioscaffold with dual bioactivities for osteochondral interface reconstruction[J]. *Biomaterials*, 2019, 196: 138-150. doi: 10.1016/j.biomaterials.2018.04.005.
- [23] Jeong JE, Park SY, Jy S, et al. 3D printing of bone-mimetic scaffold composed of gelatin/ β -Tri-Calcium phosphate for bone tissue engineering[J]. *Macromol Biosci*, 2020, 20(12): e2000256. doi: 10.1002/mabi.202000256.
- [24] Zhou X, Zhou G, Junka R, et al. Fabrication of polylactic acid (PLA)-based porous scaffold through the combination of traditional bio-fabrication and 3D printing technology for bone regeneration [J]. *Colloids Surf B Biointerfaces*, 2021, 197: 111420. doi: 10.1016/j.colsurfb.2020.111420.

(编辑 周春华, 曾曙光)



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