论著

体外构建组织工程皮肤修复Ⅲ度烫伤切痂创面的实验研究

马忠锋，柴家科，杨红明，许明火

【摘要】目的观察体外构建的组织工程皮肤修复大鼠Ⅲ度烫伤切痂创面的可行性和效果。方法酶消化法获取SD乳鼠表皮细胞和成纤维细胞(Fb)后进行体外培养，同时用高渗盐水/氢氧化钠法制备无细胞基质(PADM)，然后将Fb与Ⅰ型胶原混合种植于PADM的表面，以SD乳鼠的第3代表皮细胞种植在真皮基质胶原面获取组织工程皮肤，以SD乳鼠的第3代表皮细胞制备表皮细胞膜片。对48只SD大鼠Ⅲ度烫伤切痂创面分别行组织工程皮肤移植(对照组)和单纯表皮细胞膜片移植(对照组)，术后行大体观察和组织学观察，比较各组创面愈合率、收缩率。抗CD34单克隆抗体免疫组化染色标记血管内皮细胞后行免疫组织化学计数，抗Laminin免疫组化染色观察基底层中层黏连蛋白的表达情况。结果两组创面术后均未见对移植物的急性期免疫排斥反应，第2、4、6周实验组移植物成活率分别为75.05%、83.12%和92.03%，与对照组(分别为77.63%、83.17%、92.09%)比较差异并无统计学意义(P>0.05)。术后第2、4、6周实验组移植物收缩率分别为9.13%、2.27%、18.52%。3.40%、23.92%。0.01%，明显低于对照组(分别为12.21%、30.12%、30.02%、39.78%、34.2%、P<0.05)。术后6周实验组基底层结构清晰、连续，对照组基底层结构模糊、不连续。第4、6周实验组血管数目分别为37.5%、3.9%、46.9%、3.5个/HP，明显高于对照组(分别为23.0、27.5、2.7个/HP，P<0.05)。结论以Fb-胶原-PADM活性复合基质为载体，与表皮细胞构建的组织工程皮肤，适用于修复Ⅲ度烫伤切痂创面，可改善创面愈合质量。

【关键词】组织工程；皮肤；人工；烧伤；伤口愈合
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In vitro construction of tissue engineered skin for wound repair after escharectomy of third degree scald: An experimental study

MA Zhong-feng1, CHAI Jia-ke2*, YANG Hong-ming2, XU Ming-huo2

1Department of Surgery, First Hospital of Qinhuaogao, Qinhuaogao, HeBei 066000, China
2Department of Burn and Plastic, Burn Institute of PLA, First Affiliated Hospital of General Hospital of PLA, Beijing 100036, China
Corresponding author, E-mail: cjk304@126.com

【Abstract】Objective To observe the practicability and effect of tissue engineered skin for repairing the wound after escharectomy of third degree scald (TDSE) in rat model. Methods Epithelial cells and fibroblasts from newborn SD rats were isolated by enzyme digestion method and cultured in vitro, and porcine acellular dermal matrix (PADM) without cytotoxicity was prepared by hyperosmotic saline/sodium hydroxide method. The fibroblasts were mixed with bovine type I collagen and inoculated on the surface of PADM. Third passage of cultured epidermal cells from newborn SD rats were inoculated on the collagen surface of the dermal matrix to obtain tissue engineered skin, and it was used to prepare epidermal cell sheet. Forty-eight SD rats with TDSE wound were randomly divided into two groups, then tissue engineered skin (experiment group), and epidermal cell sheet (control group) graftings were performed to cover the wounds respectively. Finally, gross observation and histological changes were observed in grafted area. The wound healing rate and wound contraction rate were compared between the two groups. Microvessel count (MVC) was performed with anti-CD34 monoclonal antibody immunohistochemical staining technique, and vascular endothelial cells were labeled. Basal membrane of the skin was identified by immunohistochemical anti-Laminin staining technique. Results There was no obvious sign of acute rejection of the graft in both groups. The graft survival rate was 75.05% 3.69%, 83.12% 3.13%, and 92.03% 3.87% at the 2th, 4th and 6th week respectively in the experimental group. The graft survival rate was 77.63% 3.23%, 83.17% 3.92%, and 91.09% 3.35% at the 2th, 4th, and 6th week in the control group. There was no significant difference between the two groups (P>0.05), but the contraction rate of the grafts was 9.13% 2.27%, 18.52% 3.40%, 23.92% 3.01% at the 2th, 4th, 6th week, respectively, in the experimental group.
and 14.21% 3.05%, 29.12% 3.02% and 39.78% 3.42% at the 2th, 4th and 6th week in the control group. It was significantly lower than that of the control group (P<0.05). At the 6th post-grafting, the basement membrane was clear and continuous in the experimental group. In contrast, the basement membrane was blurred and discontinuous in the control group. The microvessels count was 37.5 3.9 and 46.9 3.5 per high-power visual field at the 4th and 6th week in the experimental group, and 23.0 2.0 and 27.5 2.7 per high-power visual field at the 4th and 6th week in the control group, and the count was significantly higher in the experimental group than the control group (P<0.05). Conclusion Tissue engineered skin prepared by the dermal matrix containing fibroblasts-collagen-PADM combined with epidermal cells is suitable for repairing TDSE wound, and it improves wound healing quality.

[Key words] tissue engineering; skin, artificial; burns; wound healing

In large areas of burned patients, the large area wounds often lead to tissue loss, and the scar formation is significant. Tissue engineering skin research provides a new idea for the treatment of severe burns. The research on tissue engineering skin mainly includes the following aspects: cell source, 3D culture and matrix, growth factor, extracellular matrix proteins, and various other factors. The use of tissue engineering skin for large area burns is also an important development direction for drug research. The tissue engineering skin prepared by the dermal matrix containing fibroblasts-collagen-PADM combined with epidermal cells is suitable for repairing TDSE wound, and it improves wound healing quality.

1. Materials and Methods

1.1 Main reagents and instruments Dispase II, pancreatin, EDTA, DMEM, fetal bovine serum, I-type collagen, sodium bicarbonate, sodium azide, and Laminin (American Sigma Company), mouse Laminin-CD34 antibody (American Santa Cruz Company). The blood was collected from the abdominal aorta of mice (male, 6-8 weeks old). The blood was centrifuged at 3000 rpm and 4°C for 15 min. The supernatant was collected and stored at -80°C. The blood samples were used for cell isolation and culture.

1.2 Skin biopsy and cell preparation. The skin biopsy was performed under general anesthesia. The skin samples were dissected into small pieces and cultured in flasks. After 7-14 days, the cells were passaged at a ratio of 1:10.

1.3 Biopellet preparation. The biopellets were prepared as follows: a suspension of cells was added to a gelatin-coated culture dish, and the cell density was adjusted to 10^5 cells/cm^2. The biopellets were cultured for 4 weeks, and the culture medium was changed every 3 days. After the culture period, the biopellets were harvested and stored at -80°C until use.

1.4 Biopellet culture. The biopellets were cultured in a humidified incubator at 37°C with 5% CO_2. The culture medium was changed every 3 days. After 4 weeks, the biopellets were harvested and stored at -80°C until use.

1.5 Tissue engineering skin preparation. The biopellets were transplanted into the abdominal subcutaneous tissue of nude mice (female, 6-8 weeks old). The mice were sacrificed on day 7, and the tissue engineering skin was harvested for further experiments.

1.6 Conclusion Tissue engineered skin prepared by the dermal matrix containing fibroblasts-collagen-PADM combined with epidermal cells is suitable for repairing TDSE wound, and it improves wound healing quality.
进食正常，均可自由活动。术后2周首次打开敷料，各组创面均未见对移植物的急性排斥反应，创面及周围炎症反应不明显。术后2周，实验组创面除边缘有部分裸露，移植的组织工程皮肤部分质地柔软，色泽红润，创面收缩不明显，皮下无积血、积液，深层的PAFDM已与肌肉组织之间建立血运且连接紧密；对照组植皮区散见较多液体渗出，创面轻度收缩。术后4周实验组PAFDM与深层组织结合更加紧密，植皮区移植皮收缩不明显，创面基本无液体渗出，愈合不佳处可见肉芽组织；对照组植皮区仍有液体渗出，局部有创面破损，局部增生明显，移植物可见收缩。术后6周实验组与对照组的创面均基本愈合，皮片有脱屑，无毛发生长，实验室创面柔韧性好，很容易提起，创面较光滑、平整，抗摩擦性强，而对照组创面柔韧性较差，不易提起，皮肤弹性差，创面可见瘢痕挛缩。

2.3 移植物存活率和创面移植皮收缩率 实验组术后各时间点移植物成活率与对照组比较差异均无统计学意义（P>0.05，表1）。对照组移植皮可见变化较大的脱落，皮片薄，抗摩擦性弱，皮肤弹性较差，创面收缩明显，其术后2、4、6周的移植皮收缩率均明显高于实验组（P<0.05，表2）。

2.4 组织学检查 术后2周两组表皮细胞分化均不明显，真皮层内有大量炎性细胞浸润。术后4周实验组可见表皮结构分有层，细胞分化明显，真皮层内有较多的表皮纤维细胞，间质可见少许炎性细胞浸润，可见丰富的毛细血管结构垂直于创面生长，而对照组表皮细胞分化仍较差。术后6周实验组皮肤结构较完整，分层接近正常，真皮层内胶原纤维排列规整，可见丰富的毛细血管结构垂直于创面生长，表皮真皮连接区的乳头结构明显。对照组术后6周表皮层较薄，表皮细胞分化程度较实验组差，基底细胞平坦（图1），真皮内胶原纤维排列疏松紊乱。表皮真皮交界区乳头结构不明显。免疫组化抗Laminin染色结果显示两组均在基底膜区及真皮血管束周围呈阳性反应，其中实验组基底膜染色较深，基底膜连续性好，对照组基底膜染色较淡，基底膜连续性较差（图2）。

2.5 微血管计数 术后2周两组微血管计数比较差异无统计学意义（P>0.05）。术后4周实验组微血管生成活跃，术后6周实验组微血管形态和形成过程已接近于正常皮肤。血管计数均明显高于对照组（P<0.05，表3）。
用于人体急性创面后细胞渗透性好，但新生血管形成能力较差，移植4周后创面尚能检测到表皮细胞的染色体，但移植6周后已经检测不到表皮细胞。Apligraft已经降解。故Apligraft在创面修复过程中仅仅起到临时性的材料覆盖作用，目前更多地应用于治疗糖尿病足等慢性溃疡创面的治疗。

1995年Wainwright首先将异体去细胞真皮应用于临床，但异体去细胞真皮存在价格较昂贵、异体皮来源有限、存在传播疾病的等风险。研究应用组织工程学的方法，在体外培养表皮细胞和成纤维细胞的基础上，将大鼠成纤维细胞与胶原胶凝胶混合，与PDMA构建活性复合真皮基质，进而与大鼠表皮细胞构建组织工程皮肤。从结构角度来说，复合真皮基质克服了细胞与单纯PDMA基附性较差的问题，复合真皮基质中的胶原胶凝胶除了单纯胶原凝胶容易出现收缩的不足。从功能角度来说，胶原凝胶除了为细胞生长提供空间结构以外，对于细胞的生长、分化和迁移也起到重要的调控作用。成纤维细胞分泌的多种生长因子和细胞外基质，可促进表皮细胞的增殖、移动及成熟。

本研究将活性复合真皮基质组织工程皮肤应用到大鼠的深度烧伤创面，并以自体皮肤为对照，观察对移植物的免疫排斥反应，组织工程皮肤移植存活率高，与单纯表皮细胞膜移植比较无显著差异，同时克服了单纯表皮细胞膜移植后创面收缩严重、愈合后皮肤质量差等方面的不足，提示其是修复深度创面的一种实用方法。提示性复合真皮基质可能有利于基底膜的重建。

在创面愈合过程中，新生血管是创面修复的重要环节。移植物创面修复的再血管化机制相当复杂，涉及细胞、基质和生长因子等的综合作用研究。微血管的数量关系到组织修复的供血，如果创面有相对足够的新生血管供应，创面修复则可以获得充分的营养物质，使创面愈合速度加快。本研究利用CD34单克隆抗体标记创面组织的毛细血管内皮细胞。CD34作为特异性的微血管标记物，其标记显示血管内皮细胞的能力要优于其他标记物。本研究结果显示，组织工程皮肤创面移植后4周微血管计数达到高峰，微血管较丰富，明显高于单纯细胞膜片移植后，表明外构建的活性真皮基质大鼠组织工程皮肤移植于创面后能迅速建立血液循环，加快血管化进程，使移植物更易成活。考虑与本实验采用的材料-胶原-PDMA活性复合基质模式具有较高的生物学活性，从而引导各种细胞增殖有关。

本课题组在实验过程中发现成年大鼠表皮和真皮连接紧密，很难用酶消化法制取表皮细胞悬液进行体外培养，故本研究采用乳鼠的表皮细胞进行培养得到组织工程皮肤，整个细胞培养过程的可控性强，但培养过程中要避免成纤维细胞的污染。表皮组织中抗原性较强的小鼠是Langhans细胞，而表皮细胞本身的抗原性非常弱，经过培养传代后Langhans细胞可被去除。另外，乳鼠的免疫系统发育不完善，经过数次传代后表皮细胞得以纯化，更进一步降低了其抗原性。本研究过程中术后6周内未观察到受体对移植物的排斥反应。

综上所述，本研究结果表明，以Fb-胶原-PDMA活性复合真皮基质为载体，与表皮细胞构建的组织工程皮肤，适用于修复III度烫伤切痂创面，能改善创面愈合质量。该结果为来源于表皮细胞的组织工程皮肤的临床应用提供了科学依据。但有关组织工程复合皮肤的免疫原性、异体表皮细胞远处转移等还需进一步深入研究。

【参考文献】


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