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玻璃化冻存延期回植技术在大面积皮肤撕脱伤中的应用研究*

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摘要 目的:探讨玻璃化冻存延期回植技术用于大面积撕脱皮肤伤的临床效果。**方法:**选择2015年6月至2018年6月收治的大面积皮肤撕脱伤的患者16例,根据处理方式分为撕脱皮肤玻璃化冻存二期回植组和撕脱皮肤一期回植组,每组8例患者。皮肤冻存后回植前,采用MTT法检测皮肤活力,比较两组皮片回植后的成活率、感染率、平均住院时间。**结果:**玻璃化冻存组皮肤冻存后平均活力为 $92.56\% \pm 7.20\%$,皮片成活率为 $81.74\% \pm 5.78\%$,感染率12.5%,平均住院时间 33.13 ± 3.91 d;一期皮肤回植组皮片成活率 $21.91\% \pm 5.28\%$,感染率100%,平均住院时间 47.50 ± 1.93 d。玻璃化皮肤保存组皮片成活率、感染率、平均住院时间均优于一期皮肤回植组($P < 0.05$)。**结论:**皮肤玻璃化冻存延期回植技术用于无法一期回植皮肤撕脱伤的临床效果较好。

关键词:皮肤撕脱伤;玻璃化皮肤保存;延期回植技术

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A Study on the Application of Delayed Back Grafting Technique with Vitrification Cryopreservation Skin in the Treatment of Serious Avulsion Injury*

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ABSTRACT Objective: To observe the clinical efficacy of delayed back grafting technique with vitrification cryopreservation skin in the treatment of serious avulsion injury. **Methods:** Patients suffered from serious skin avulsion from June 2015 to June 2018 were divided into two groups: the vitrification preservation group and the one-stage replanting group according the management method, 8 patients in each group. The cells viability of the skin before replanting after cryopreservation were evaluated using MTT. The skin survival rates, infection rates, average in-hospital time were compared between the two groups. **Results:** The average cells viability of the skin before replanting after cryopreservation was $92.56\% \pm 7.20\%$. The skin survival rates was $81.74\% \pm 5.78\%$. The infection rates was 12.5%, and the average in-hospital time was 33.13 ± 3.91 d. The skin survival rates, infection rates, average in-hospital time in the one-stage replanting group were $21.91\% \pm 5.28\%$, 100%, 47.50 ± 1.93 d. There is difference between the groups in the skin survival rates, infection rates, average in-hospital time ($P < 0.05$). **Conclusion:** The clinical effect of delayed back grafting technique with vitrification cryopreservation skin is good in the treatment of serious avulsion injury that skin can not be replanted one-stage.

Key words: Skin avulsion injury; Skin cryopreservation technique; The delayed back grafting technique

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前言

皮肤撕脱伤是一类临床上常见的创伤^[1-3],常伴有创面污染、撕脱组织坏死、骨折、失血性休克等,严重时需截肢,甚至危及生命。目前的常规修复方法为保留有血运的皮瓣,切除无血运的皮瓣,反取皮回植^[4,5],但往往由于创面可能存在的潜在污染,导致一期的反取皮片容易坏死、感染,仍需二期从正常部位取皮,覆盖创面。有些患者全身情况差,一期无法耐受手术,必

须二期手术^[6],导致撕脱皮肤无法利用。因此,这种修复方法不仅浪费了撕脱的皮肤,又造成身体其他部位的损伤,存在较大缺陷。

将撕脱皮肤应用皮肤冻存技术进行短期保存^[7,8],待适宜手术时再将其进行回植,为解决这一临床难题提供新的途径。超低温迅速冻存使组织形成一种类似玻璃样的物质,细胞停止新陈代谢,理论上可无限期保存组织^[9,10]。课题组自2015年6月至2018年6月将皮肤玻璃化冻存延期回植技术用于皮肤撕脱

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伤的治疗,取得了较好的效果,现报道如下。

1 材料与方法

1.1 纳入标准、分组

纳入标准:患者年龄 <60 岁,皮肤撕脱面积 $\geq 100 \text{ cm}^2$,撕脱皮缘无活动性出血,没有合并骨、肌腱、神经、血管外露。

分组:根据皮肤处理情况分为低温保存组和一期回植组。

本实验实施前已通过中国人民解放军空军军医大学第二附属医院伦理委员会批准(批准编号 TDLL201704-20)。

1.2 方法

1.2.1 撕脱皮肤处理 根据郭树忠等^[1,12]方法,将撕脱皮肤用生理盐水清洗、用 0.1% 苯扎溴铵浸泡 15 分钟消毒,去除碾挫伤较重部分,剩余皮肤用鼓式取皮机反取皮,修成 0.3-0.5 mm 厚皮片。

1.2.2 皮片玻璃化冻存处理 根据盛志勇等^[13]的方法,将皮片经过冻存液(成分为:20%DMSO、6%丙二醇、Kreb Ringer 磷酸缓冲液)浸泡 30 分钟,塑封,放于液氮中保存。复温时,将冻存皮从液氮中取出后立即放入无菌 40 °C 水浴中,不超过 2 分钟,皮片变软立即拿出。

1.2.3 创面处理和植皮 根据郭树忠、侯春林教授^[11,14]所选的方法对皮肤撕脱伤创面早期彻底清创。一期回植组将皮片回植

于创面,玻璃化冻存组先常规换药,待创基适宜植皮时,再进行皮肤回植。两组皮片回植后均加压包扎固定,1 周后打开换药、拆线。

各组患者如回植皮片不足以覆盖创面,则从患者背部或大腿取皮补充,皮片打包或加压包扎、局部制动。

1.3 观察指标

(1)玻璃化皮肤保存后皮肤活力测定(MTT 法^[15]);(2)皮片的成活率;(3)皮片感染率;(4)平均住院时间。

1.4 统计学处理

实验数据输入 SPSS 20,计量资料均数比较采用 t 检验,两样本率的比较采用 χ^2 检验,以 $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 玻璃化皮肤保存后皮肤活力、两组皮片的成活率、感染率、平均住院时间比较

如表 1 所示,玻璃化皮肤储存组复温后皮片活力平均为 $92.56\% \pm 7.20\%$,玻璃化皮肤储存组皮片成活率 ($81.74\% \pm 5.76\%$) 高于一期植皮组 ($21.91\% \pm 5.28\%$),感染率 (12.5%) 低于一期植皮组 (100%),平均住院天数 (33.13 ± 3.91) 小于一期植皮组 (47.50 ± 1.93)。

表 1 玻璃化皮肤保存后皮肤活力、两组皮片的成活率、感染率、平均住院时间比较

Table 1 Comparison of the skin vitality after vitrification cryopreservation, the skin graft survival rate, the infection rate, the average in-hospital time between the two groups

Groups	Skin Vitality after Cryopreservation	Skin Graft Survival Rate	Infection Rate	Average In-hospital Days
ORG	--	$21.91\% \pm 5.275\%$	100%	47.50 ± 1.927
VPG	$92.56\% \pm 7.20\%$	$81.74\% \pm 5.757\% *$	12.5%*	$33.13 \pm 3.912 *$
t/ χ^2	--	t=7.662	$\chi^2=7.662$	t=3.296
P	--	$P < 0.0001$	$P = 0.0014$	$P = 0.0053$

Note: ORG: One-stage Replanting Group, VPG: Vitrification Cryopreservation Group.

2.2 典型病例

病例 1 患者田某某,女,36 岁,车祸致左下肢大面积撕脱伤,伤后经清创+封闭负压引流+撕脱皮肤玻璃化冻存,使大部分撕脱皮肤得以再利用,减少了取皮面积,效果较好。

病例 2 患者王某,7 岁,车祸致双下肢大面积撕脱伤,急诊给予清创,撕脱皮肤修成全厚皮后回植;术后 3 天回植皮肤全部坏死,创面经多次清创换药,待新鲜后再次植皮后得以康复。

3 讨论

3.1 皮肤玻璃化保存延期回植技术可解决不能一期修复患者撕脱皮肤再利用的临床难题

皮肤撕脱伤是临床上常见的疾病^[1-3],常规治疗方法是清创后,撕脱皮肤直接缝合或反取皮回植覆盖创面^[3,4]。郭树忠等^[11,12]认为治疗的难点是:(1)如何准确评估撕脱皮肤血运情况和创基情况;(2)如何提高皮肤(皮片)的成活率。目前大量研究^[16-19]显示根据皮肤的颜色、温度、充盈反应、出血情况等指标主观评判,准确评估撕脱皮肤血运情况非常困难,尤其是受伤早期。根据

郭树忠等^[11,12]研究结果,如果撕脱组织完全与身体分离,或不完全分离但皮肤无血运或撕脱皮肤真皮下血管网已栓塞,需将撕脱皮肤切取后,反取皮修成中厚或全厚皮回植。本实验也显示在撕脱皮肤毁损组,撕脱皮肤血运判断困难,在伤后就诊时间长的病例,撕脱皮肤中可见大面积血管栓塞。此类患者无法直接直接缝合,需进行反取皮修薄后回植。

但是在临床工作中,我们经常遇到患者创基严重污染,需进行多次清创,或者患者伴有严重的复合伤,无法实施手术。Lenarz CJ^[20]等研究发现高能损伤或骨折伴广泛组织损伤的创面,清创后的细菌培养阳性率仍然高达 10%,Kudsk 等^[21]报道了利用原位缝合法治疗肢体撕脱伤,其结果是大面积的皮肤坏死及创面感染。这类患者应用常规方法往往导致皮片坏死、感染等^[22],如果将撕脱皮肤丢弃,二期从正常部位取皮,不仅浪费了患者的皮肤资源,又造成二次损伤。常规治疗方法已无法满足此类临床治疗的需要。

针对以上问题,我们利用玻璃化皮肤储存的方法,将撕脱皮肤修薄后,经抗冻处理,进行低温保存,待患者生命体征平



图1 玻璃化冻存组典型病例

Fig. 1 A Typical Case of Vitrification Cryopreservation Group

Note: Vitrification Cryopreservation Group: Tian XX, Female, 36Y, had the extensive avulsion injury in the left lower limb by the accident.

- ① Debridement + VAC were treated emergency. The avulsion skin was thinned to 0.6mm graft,
vitrification cryopreserved and replanted after rewarming in 24 days: ② The rewarmed skin graft,
③ Replanting Surgery, ④ The skin graft survival was good in 10 days after replanting surgery. ⑤ 1 month after replanting surgery.

稳、创基新鲜时,再进行皮肤回植。以此,可以有效保存皮肤活力,减少患者皮肤的浪费,减轻再次取皮带来的二次创伤。研究结果显示玻璃化冻存组皮肤成活率、感染率、平均住院时间明显优于一期回植组,证实了这一点。

3.2 玻璃化冻存技术为延长皮肤冻存时间提供了保障

常规的 4℃、-20℃ 皮肤冻存仅能局限于短期内保存,时间过久皮肤活力就会明显下降,无法满足临床应用,4℃ 保存方法有盐水纱布法、营养液法、B 培养液法^[23],但储存时间大多无法超过一周^[24,25];针对 -20℃ 皮肤冻存^[26,27]的研究显示虽然加入二甲基亚砜、丙二醇等抗冻剂,甚至抗氧化剂后,但也只能在两周内保持皮片活力。临床上,大量大面积皮肤撕脱病人由于创面污染重无法一次彻底清创,需反复多次清创;并且部分患者还合并其他脏器损伤、骨折等等,都会影响二期创面修复开始的时间,大部分该类患者往往需要 3 周左右的准备时间,才能进行二期修复,这就要求皮肤冻存必须能够保持至少 3 周的活力,而上述低温冻存方法无法达到。

一般的组织冻存会在细胞内产生冰晶,冰晶造成细胞损

伤,而快速的深低温冻存,细胞内冰晶无法形成,而是成为一种玻璃化状态,不会造成明显的细胞损伤。因此,玻璃化冻存被认为是保存组织的最好方法,理论上可以无限期保存组织^[28-32]。根据解放军总院第一附属医院烧伤科和陆军军医大学烧伤研究所在玻璃化冻存皮肤方面所作出的大量研究^[13,33],目前主要针对异体皮的储存,玻璃化冻存方法可靠,但未有关于大面积撕脱皮肤的玻璃化冻存研究,本课题组结合现有条件采用抗冻效果较好的 20%DMSO、6%丙二醇、Kreb Ringer 磷酸缓冲液作为本实验的抗冻液配方,将无血运的撕脱皮肤修成皮片,快速放入液氮中,进行玻璃化冻存,结果显示该方法可有效保持皮肤活力,二期回植时,皮片成活率较高,且保存时间较长,能够满足临床二期植皮的需要。

3.3 正确的冻存与复温操作是实施皮肤低温保存延期回植技术成功的保证

我们对冻存后皮肤活力进行检测,发现玻璃化冻存在一定时期内能够保持皮肤较高活力(92.6%)。回植后成活率达 81.7%左右,可以满足临床的需要。考虑活力下降的原因可能是皮肤

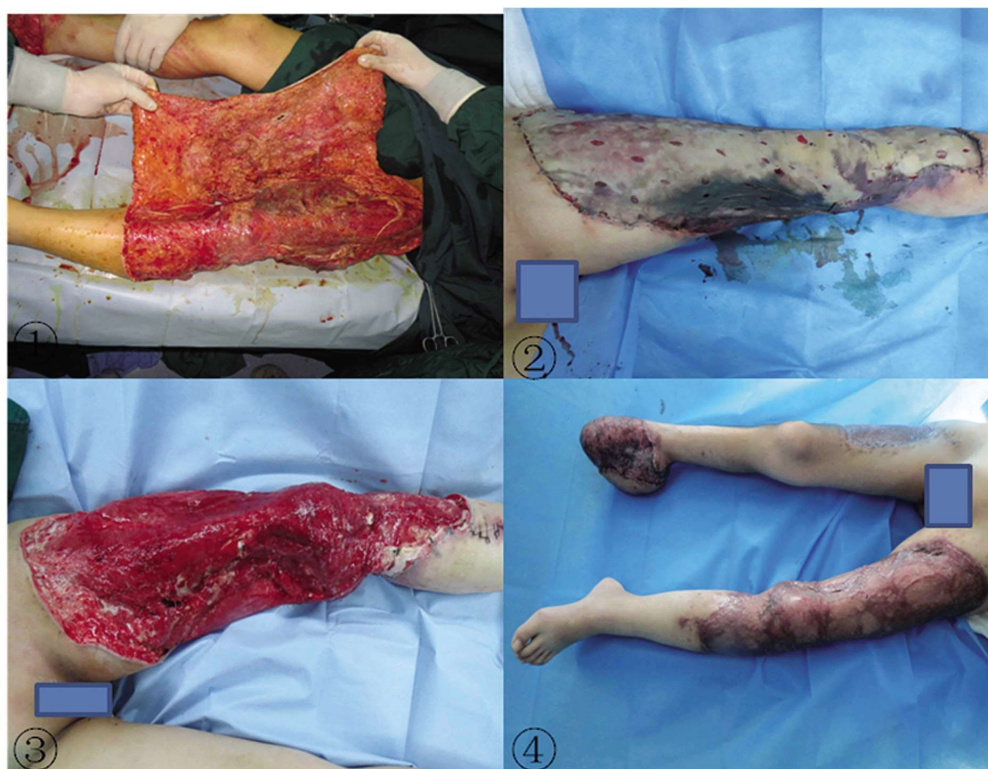


图2 一期回植组典型病例

Fig. 2 A Typical Case of One-stage Replanting Group

Note: Wang XX, 7Y, had the extensive avulsion injury in the two lower limbs by the accident, whose avulsion skin was replanted after thinned to full-thickness graft. ① The avulsion skin, ② The replanted skin necrosis was shown after 3 days. ③ The wound was new after serial of debridement and dressing changing on the 13th day. ④ The wound was cured 1 month after repaired by grafting new skin.

冻存和复温操作时,皮肤经室温至 -196°C 或从 -196°C 至室温这两个过程中造成细胞损伤,而细胞在玻璃化状态下的损伤较少。

皮肤的冻存与复温是皮肤保存活力的两个关键步骤,有以下注意事项需引起重视:(1)低温皮肤保存技术需严格按照杨宗城^[34]等所述操作,包括抗冻液配置、快速降温、快速复温、消毒等,如操作不当,反而会冻伤或污染皮肤,降低活性,影响成活率;(2)皮肤上如有碾挫伤痕迹,证明此处皮肤内组织存在碾挫伤,最好将其去除,否则不宜成活;(3)皮肤回植后仍需打孔,并加压或打包包扎、患肢制动。

尽管低温皮肤储存延期回植技术取得了较好的疗效,但其仍有严格适应症,如果合并骨、肌腱、神经、血管外露,该方法不适用,仍需要考虑应用局部或远位组织瓣修复。

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