·临床研究·

烧伤皮肤再生疗法与创面愈合的机制

徐荣祥，萧 摩

【摘 要】 目的：从分子、细胞和整体临床治疗三个层面，解析烧伤皮肤再生疗法在烧伤创面愈合中的作用机制；论证皮肤再生医疗技术的理论基础和临床实用价值。方法：从烧伤创面坏死组织液化排除（祛腐）和创面的原位皮肤再生修复（生肌）两个关键环节，分析规范应用烧伤湿润暴露疗法和湿润烧伤膏（MEBT/MEBO）在烧伤创面愈合过程中的重要作用和临床疗效。结果：烧伤创面的生理性原位皮肤再生修复需要具备三个条件：一是烧伤创面生理湿润环境的形成；二是原位培养角蛋白 19 型干细胞的再生物质和组织学基础；三是规范的皮肤再生医疗技术是创面生理性再生愈合的保证。结论：潜能再生细胞理论和原位干细胞培植再生修复技术是烧伤皮肤再生疗法的理论基础和实用医疗技术。严格实施皮肤再生医疗技术的操作程序和规范的治疗方法，对烧伤创面愈合有明显促进作用，能使深度烧伤创面达到生理性再生愈合。

【关键词】 烧伤；再生疗法；潜能再生细胞；原位干细胞培植技术；纤维隔离膜；MEBT/MEBO

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烧伤皮肤再生疗法是以创面皮肤原位再生为技术核心，以深度烧伤无瘢痕愈合为判断标准的烧伤皮肤再生医疗技术，其主要机制是通过无损伤的液化排除坏死皮肤组织（祛腐），为烧伤创面创造一个生理的湿润环境，同时激活潜能再生细胞，促进创面以原位干细胞再生修复方式达到烧伤创面生理性愈合（生肌）。多年的基础与临床研究证实，MEBT/MEBO 促使烧伤创面完成坏死组织的液化和新生皮肤的再生，是极其复杂的生理过程。本文试从分子、细胞和全身系统治疗三个层面对深度烧伤创面愈合机制进行分析，以论证烧伤皮肤再生疗法达到创面生理性愈合的必然性。

烧伤创面的皮肤再生修复必须在创面坏死皮肤组织清除的情况下方可完成，如果以外科切除（或削除）方式去除坏死皮肤组织，势必将部分处于间生态的组织（还未完全坏死、尚存生机的组织）切除，同时也切除了残存于创基的大量含有外胚层基因的信息细胞和潜能再生细胞。由于这些细胞是烧伤创面再生修复的最基本的物质基础，故临床治疗原则应是尽量保护好这些组织细胞，使其充分发挥在原位转化干细胞再生修复烧伤创面过程中的作用，这也是烧伤皮肤再生疗法能保障烧伤创面生理性无瘢痕修复的理论基础。

一、烧伤创面坏死组织液化排除与实施方法

烧伤创面坏死皮肤组织的液化排除是烧伤创面原位再生修复的必备条件，无损伤的液化排除烧伤创面坏死皮肤组织，必须在创面外用湿润烧伤膏（MEBO）并规范应用皮肤再生医疗技术下完成。其基本治疗原则是最大限度地保留残存于创基且具有活力的组织细胞，使那些间生态组织在生理的湿润环境下复苏，为创面的再生修复创造条件。

（一）创面坏死组织液化排除过程

坏死组织液化是在 MEBO 的作用下，使固态的烧伤创面坏死组织转化为液态，并从创面上排除的过程。欲诠释烧伤创面坏死组织液化过程之前，应首先了解 MEBO 特有的（低熔点）框架剂型的药理作用。框架剂型是随着温度变化而改变的可变剂型，在常温下呈软膏形态，外涂于烧伤创面后，由于皮肤温度的

【作者单位】中国烧伤创疡科技中心，北京 100053

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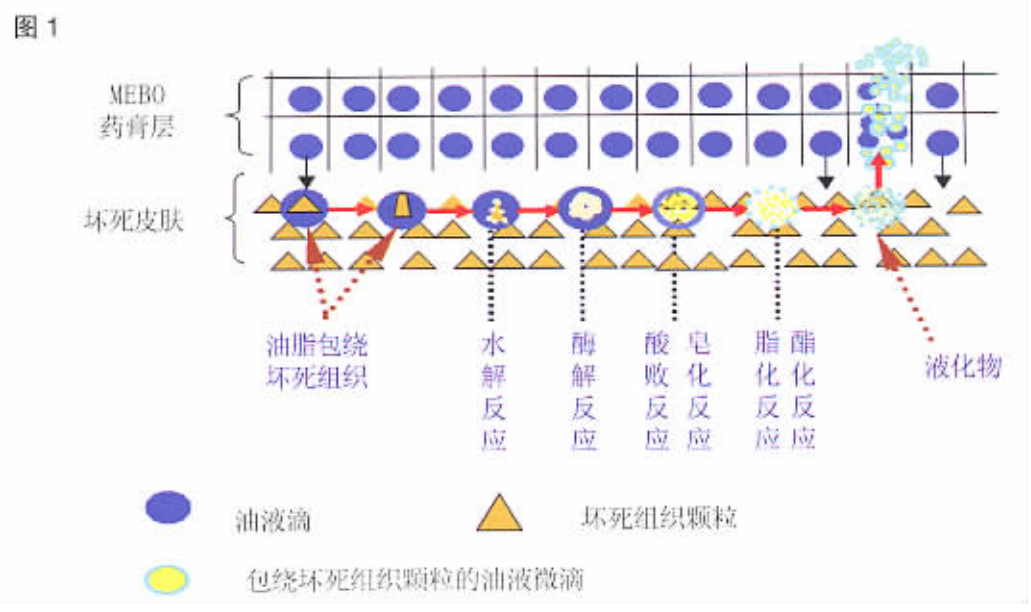
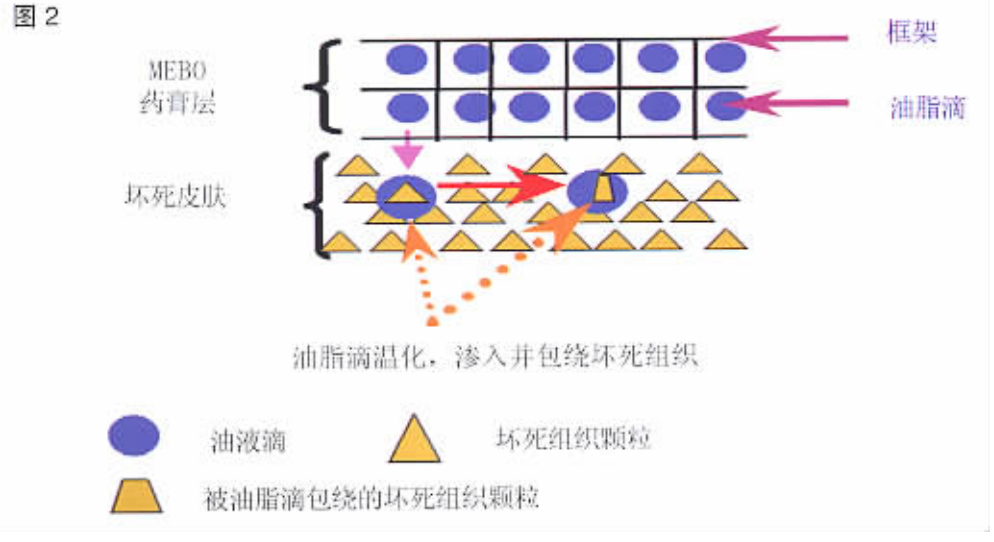
温化而成为液态，1 毫米厚的药膜形成两层，暴露在空气的表面层为膏态，接触创面层为液态。由于 MEBO 具有亲脂性，液态部分的药物与创面坏死组织发生反应变性，失去了亲脂性，并与创面的渗出物、液化物相混合后，向药膜外移动以至冲破药膜排出。上层药物在皮温扩散的温化下，持续供给液层，液层药物又不断与创面坏死层发生水解、酶解、酸败、皂化、脂化和酯化等多种生物化学反应，循环往复自动引流是促使创面坏死皮肤组织从固态转化为液态的主要机制。新鲜的 MEBO 药膏不断供给创面，在发挥无损伤的液化排除坏死组织功能的同时促进创面皮肤再生修复。烧伤创面坏死组织液化过程如下：见图 1

图 1



1. 温化透入包绕坏死组织：首先是 MEBO 中具有油脂性质的基质在创面温度的温化下，由固态的软膏剂型转化为液态，温化的油滴渗入创面，将坏死皮肤组织分割为颗粒并将其包绕，从而启动坏死组织连续发生系列的生化反应。见图 2

图 2



2. 水解反应：一种化合物在水的作用下分解的反应称水解反应。MEBO 油滴渗透入创面，将坏死皮肤组织颗粒分割包绕，坏死组织细胞中残存的水分在 MEBO 油滴的包绕下与坏死皮肤发生分解反应，从而

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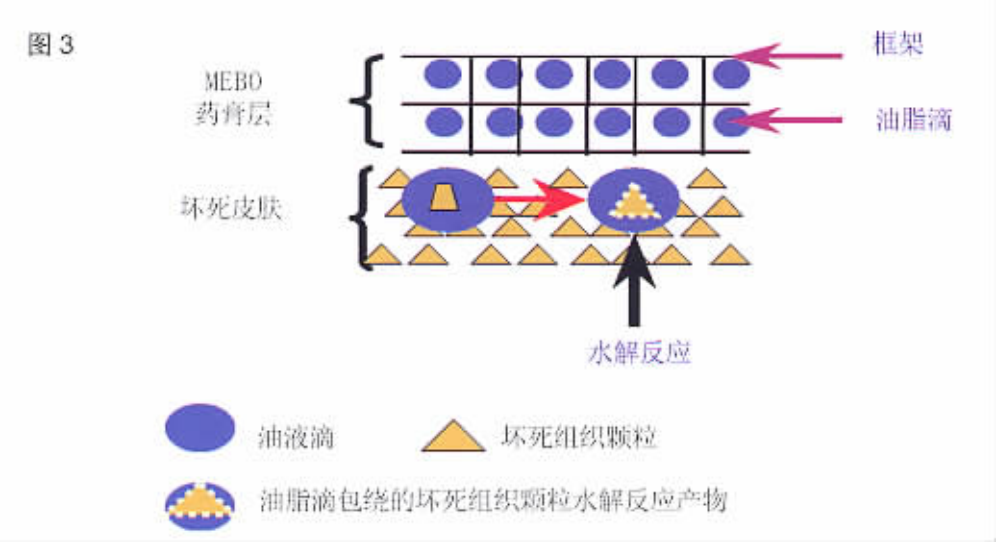
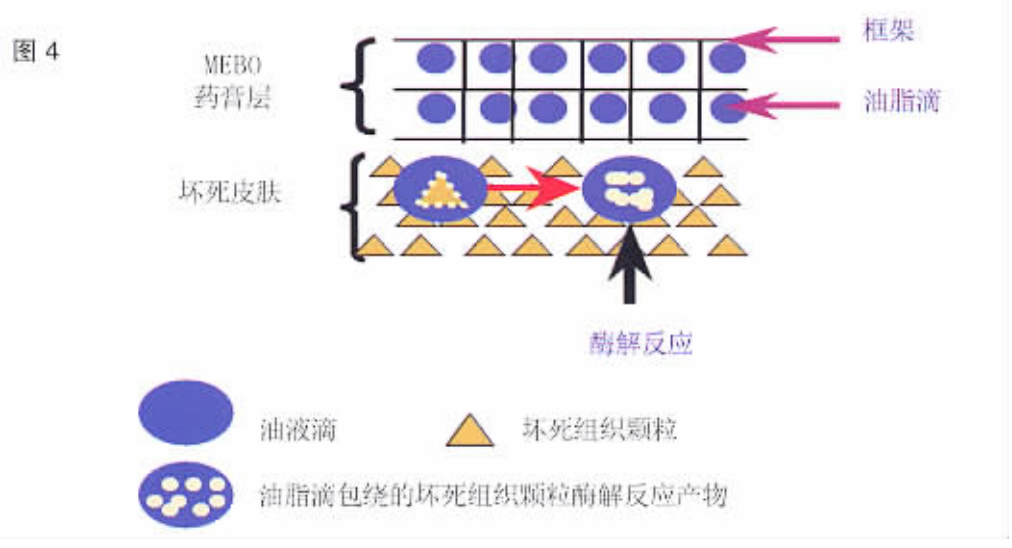
启动了创面坏死组织液化排除的系列生物化学反应。见图 3



3．酶解反应：创面坏死组织细胞中包含着多种酶原。酶原是酶的无活性前体，在水解反应发生后除去若干肽分子激活酶原，使坏死组织发生酶解反应，从而使创面坏死组织细胞中的各种蛋白质、脂肪、碳水化合物等大分子有机物质，在被激活的多种酶的作用下进一步分解为小分子物质，使创面的坏死组织变为分子状态。见图 4



4. 酸败、皂化反应：酸败反应的含义是坏死组织中的蛋白质、脂肪组织经过上述水解、酶解等生物化学反应，分离出氨基酸、脂肪酸、产生醛酮类氧化物。所产生的这些酸性化合物一般由氢原子和酸根组成，在水溶液中可产生氢离子的化合物。组织坏死和烧伤后机体处于高代谢状态时也产生大量酸性代谢产物，这些有机酸分解或化合反应后产生中性的盐和水。皂化反应指脂肪在碱性溶液中水解，产生甘油、脂肪酸等。

烧伤创面通过 MEBO 的治疗作用可以使创面组织变性坏死，产生大量的组胺、缓激肽、乳酸、自由基等酸性物质（统称烧伤毒素），并发生分解反应，从而减轻了对创面的直接损伤和毒素吸收后对机体多器官造成的损害。总之，酸败、皂化反应的结果使烧伤创面坏死组织分解成中性的组织颗粒，且便于液化

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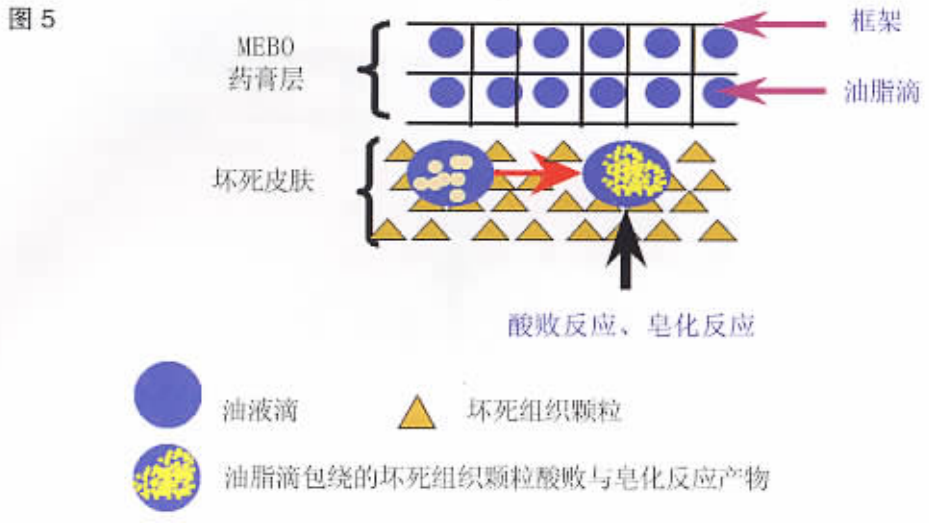
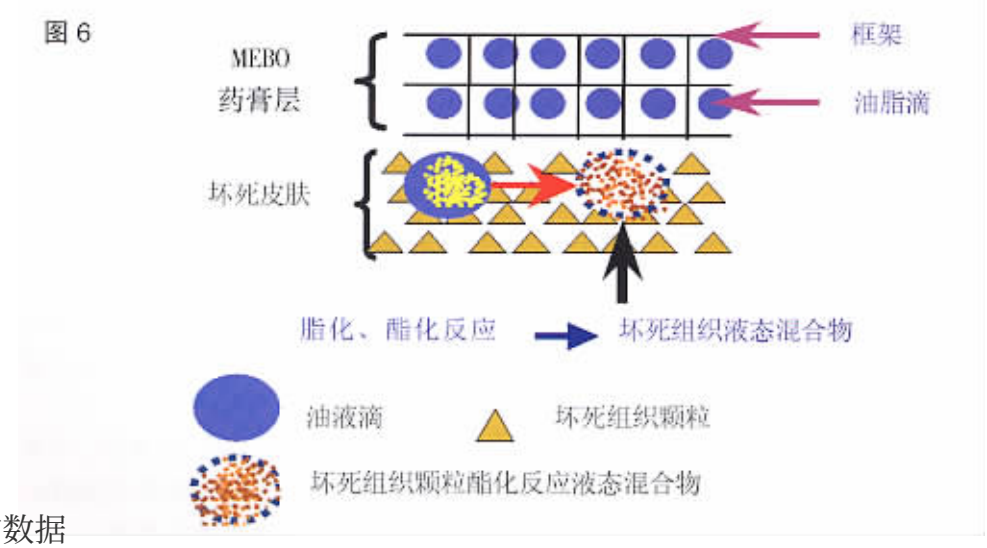
排除，可有效地保护创面，减轻烧伤毒素吸收对机体的损伤。见图 5



烧伤创面坏死组织在 MEBO 的作用下，经过水解、酶解、酸败、皂化等化学反应后，产生无损伤的液化排除作用，同时，创面的皮肤再生修复程序被启动，其中脂化和酯化反应是创面液化的重要生物化学过程。

5．脂化、酯化反应：MEBO 属于网状框架剂型，由蜂蜡包裹油脂组成，其中芝麻油含有丰富的亚油酸成分（属于不饱和酸）可以和上述反应所产生的创面液化物中的固醇类物质、醛酮类氧化物、脂质、类脂质等物质发生分解反应，这个过程为“脂化反应”。此后，MEBO 中的亚油酸还可继续与上述物质结合而成为酯，谓之“酯化反应”。

创面坏死组织液化过程中可使酯类物质降解为酸，其逆反应就是在酯酶（生物酶）的催化作用下使酸变成酯类液态混合物。通过这些反应过程，达到降低坏死组织中酸性物质的毒性，减轻对创面生理湿润环境的破坏和对创面组织的继续损伤。同时，酯类化合物对皮肤创面活组织有一定的保护作用，可以促进创面愈合并养护初愈的新生皮肤组织。由此可见，脂化和酯化反应是相继发生的可逆性生物化学反应。其中芝麻油（亚油酸）成分与创面坏死组织液化物发生的分解反应，既是 MEBO 能为创面营造生理湿润环境的主要基质，也是促使分解和产生坏死组织液化过程的主要成分，最后使创面坏死组织形成酯类液态混合物，其毒性和局部刺激性均已显著下降而且便于清除，保证了烧伤创面的皮肤原位再生修复程序的完成。见图 6



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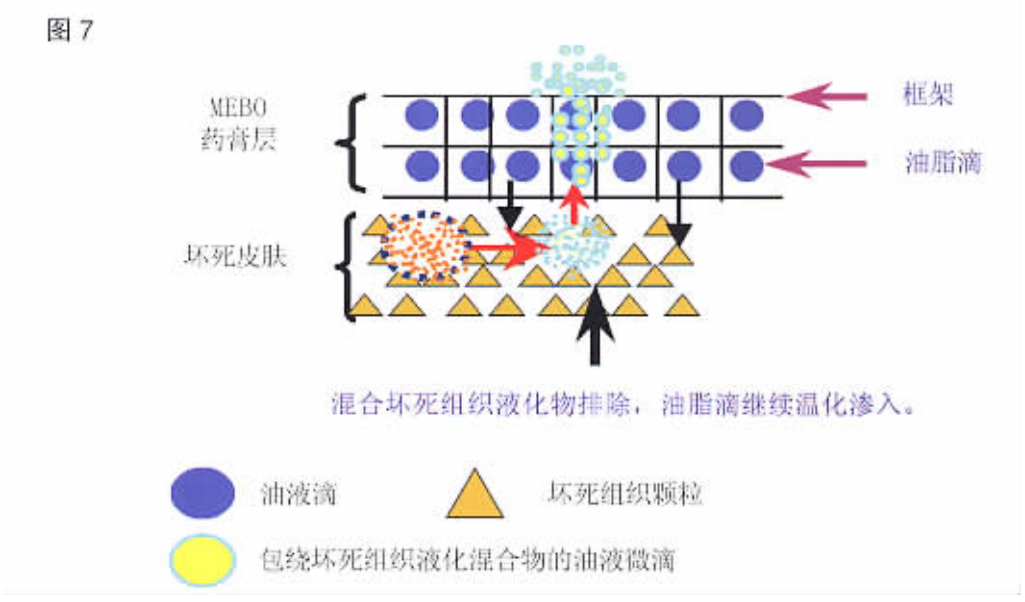
MEBO 在烧伤创面发生的诸多生物化学反应，使坏死的皮肤组织颗粒形成液态混合物，通过 MEBT 的局部创面换药技术，使坏死的皮肤组织在无损伤的情况下清除，保证创面的再生修复实现无瘢痕愈合。但应指出，MEBT/MEBO 的作用机制和临床疗效的发挥，必须正确和规范地应用皮肤再生医疗技术的前提下方可以完成，包括创面的局部处理和全身性烧伤再生医学疗法（Burn Regenerative Therapy BRT）的系统治疗。见图 7

图 7



（二）创面液化期的技术实施方法

MEBO 在烧伤创面所发生的诸多生物化学反应，使坏死的皮肤组织颗粒形成液态混合物，通过 MEBT 的局部创面换药技术，使坏死的皮肤组织在无损伤的情况下清除，保证创面的皮肤再生修复是烧伤皮肤再生技术的重要步骤。临床实践证明，在坏死组织液化排除后，创面的表面所形成的一层透明膜类似于角膜的纤维隔离膜，保护好这层薄膜对创面的生理性愈合至关重要。

创面坏死组织的液化表现不同于创面感染，感染是指尚存有生机的组织在致病菌的作用下产生炎症反应并导致化脓的过程，其特点是局部出现红、肿、热、痛和功能障碍。而液化是在 MEBO 作用下使已坏死的上皮组织逐渐由固态转化为液态的自然演变过程，它不需细菌参与，为无损伤的排除过程，其临床表现是在创面上形成一层乳白色或乳黄色均匀细腻的半流体的液化物，通过换药时的操作无损伤的清除。在换药技术上要掌握以下原则：

1．早期用药：在伤后 4 小时以内用药最佳，目的是抢救烧伤创面的瘀滞带组织，防止创面发生进行性坏死，保证疗效。

2．全程用药：在治疗过程中不间断用药，不能中途改用其它疗法和其它药物，禁用干燥、收敛类药物，也不用消毒剂直接涂于创面，更不要用水剂清洗创面。

3．规范创面换药技术：创面液化物必须每 4 小时左右清除一次，在清除过程中要作到不使病人疼痛、不宜出血和不损伤正常组织为原则，关键是保护好贴覆于创面的纤维隔离膜，以“三不原则”保证创面生理性再生修复。

4．创面处理：一是要做到“三个及时”，即及时清理液化物，及时清理坏死组织，及时供药；二是要达到“三不积留”，即创面上不积留坏死组织、不积留液化物、不积留多余的 MEBO 药膏。

治疗大面积烧伤患者遵循以上原则是必要的，除保证创面尽快愈合外，还直接关系到全身情况的变化和恢复，减少创面细菌数量和坏死组织所产生的炎性介质及毒素的吸收，会有效地改善全身情况，减

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轻全身中毒症状。

二、烧伤创面的皮肤原位再生修复机制

烧伤创面皮肤的原位再生修复需要具备三个条件：一是烧伤创面生理湿润环境的形成；二是原位培养皮肤角蛋白 19 型干细胞的再生物质和组织学基础；三是规范的皮肤再生医疗技术，保证创面皮肤的原位再生修复。

（一）烧伤创面生理湿润环境的形成

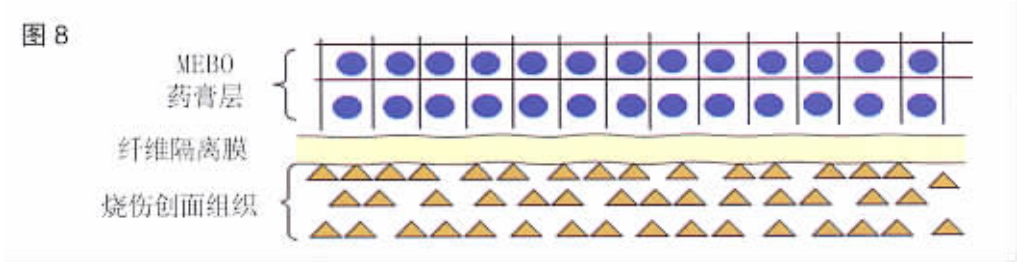
在应用烧伤皮肤再生疗法使烧伤创面坏死组织液化排除的过程中，创面已经形成了生理湿润环境。坏死组织液化排除基本完成之后，在创面的表面形成一层透明膜，它类似于角膜的纤维隔离膜。这层膜的存在是证明烧伤创面生理湿润环境形成的标志，也是原位干细胞再生皮肤组织器官的生命保护膜，保护好纤维隔离膜是烧伤创面皮肤生理再生修复的保证。

实验证明，具有 MEBO 和纤维隔离膜保护的烧伤创面，组织水分蒸发量与正常皮肤相近似，说明它替代了正常皮肤组织的保护和呼吸功能，即不出现因创面干燥暴露而发生的大量水分丢失，也不发生象凡士林完全封闭创面所造成的组织窒息和浸渍。

1．纤维隔离膜的成分与形成：MEBO 与烧伤创面渗出物的相容性较强，在创面坏死组织液化过程中所形成的纤维隔离膜，其成分为 MEBO 促使坏死组织液化过程中，经系列生化反应所产生的酯类物质和创面渗出血浆蛋白形成的酯蛋白结合物。该膜紧密贴覆于创面表层，保持创面的生理湿润环境，实现创面再生修复。然而，只有规范的应用 MEBT/MEBO 方能在创面上形成纤维隔离膜。

2．纤维隔离膜的特性与功能：由于纤维隔离膜由特殊成分组成，使其具有“半透膜”的特性与功能。该膜形成于创面与 MEBO 之间，能使隔离膜两侧的水相（创面组织）和油相（MEBO）之间形成渗透递度，使各种物质成分以离子对流方式发生交换，创面的代谢产物可通过渗透作用排泄到隔离膜的外表层。同理，MEBO 中的营养成分和具有生物活性的药物成分，也会通过隔离膜渗透到创面深层，充分发挥其促进皮肤组织再生修复的生理和药理作用。见图 8（纤维隔离膜的示意图）

图 8



3．纤维隔离膜的临床意义：烧伤创面皮肤原位再生修复的全过程是在纤维隔离膜的保护下和规范应用 MEBT/MEBO 完成的，只有在纤维隔离膜存在的状态下，创面深层的微循环结构才能以生理的毛细血管树的架构形态再生，以保障为原位干细胞再生形成的原始皮肤胚胎基和皮岛输送营养，使其发育扩展愈合创面。所以，在创面换药、清理等操作中要特别注意保护好纤维隔离膜的完整性，以持续供药方式始终保持创面的生理湿润状态，切忌干燥或浸渍。这也是坚持“三不损伤”、“三不积留”原则治疗与护理创面的原由所在。

（二）皮肤干细胞的原位培养

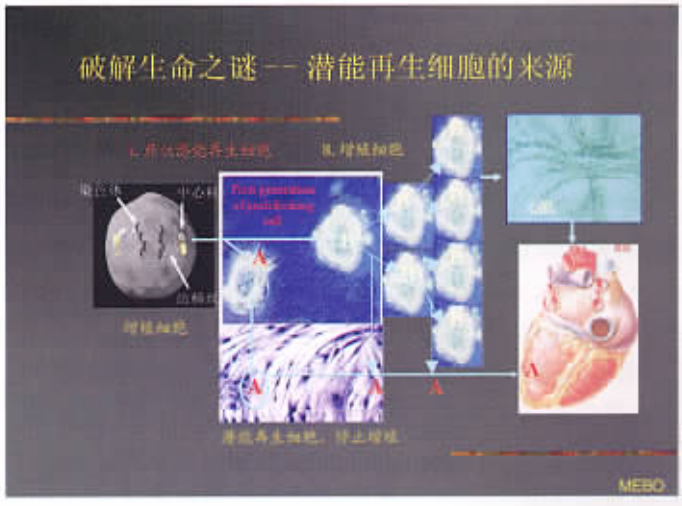
烧伤创面皮肤原位再生的组织学基础是潜能再生细胞（PRCs）的发现和原位干细胞培植再生修复技术的应用。原位再生皮肤器官的潜能再生细胞主要是皮下组织细胞分化的角蛋白 19 型皮肤干细胞，深达骨骼的烧伤可将骨髓细胞通过原位骨钻孔引入皮下，分化为角蛋白 19 型干细胞原位再生修复创面。

万方数据

在正常人体组织器官新陈代谢的生命过程中，组织细胞会不断的凋亡、退化、损伤、坏死，同时又有新生的细胞去增殖补充，以维持其组织器官的架构和生理功能。机体恢复所丧失的局部组织细胞的过程称为再生，而生理性再生是正常细胞的新老更换。人类的这种再生功能是固有的本能，是与生俱来的。关于生命延续之迷的破解以及发生规律，历经十年的精心研究，其成果于 2002 年公布于众。应用原位干细胞培植再生修复医疗技术，使烧伤创面皮肤原位再生的源头是从潜能再生细胞（PRCs）开始的。这一生命规律的揭示，为烧伤皮肤再生疗法中创面的生理性再生修复作出了科学的诠释。

1．潜能再生细胞（potential regenerative cell PRCs）的定义：潜能再生细胞是与组织细胞在一起的、有潜能的、静态的及原位性的和有干细胞再生功能的细胞。见图 9

图 9



干细胞在分裂过程中有纺锤丝出现，故称有丝分裂。分裂期间染色体进行自我复制，复制后的染色体又均等地分配至两个子细胞中，其中一部分细胞继续分裂、增殖，而另一部分细胞不再继续分裂、增殖，潜伏在组织中成为潜能再生细胞。

人类组织器官中的潜能再生细胞是在组织器官发育形成的各个时期，由原始和多能干细胞增殖时产生的。这些细胞以普通细胞形式存在，当组织器官的细胞发生凋亡、退化、损伤坏死时，会原位启动自身的增殖功能，再生复制新的细胞，及时补充器官中的细胞和组织功能的空缺，恢复器官的结构和功能，保障器官组织功能的持续。只要人体组织器官的这种再生功能发挥正常，就能维持整体生命的平衡。

2．烧伤创面皮肤原位再生修复的概念：即皮肤再生医疗技术促进烧伤创面再生修复的全过程，是将创面置于一个特殊的生理湿润环境中，并持续供给细胞生长所需的生命物质（MEBO），在创面原位激活启动潜能再生细胞，促进其形成干细胞并在原位持续分裂再生，通过诱导、调控、分化为各种组织干细胞；再经过干细胞之间的培植、连接，组合为成体组织，从而完成烧伤创面原位再生修复皮肤器官的过程。

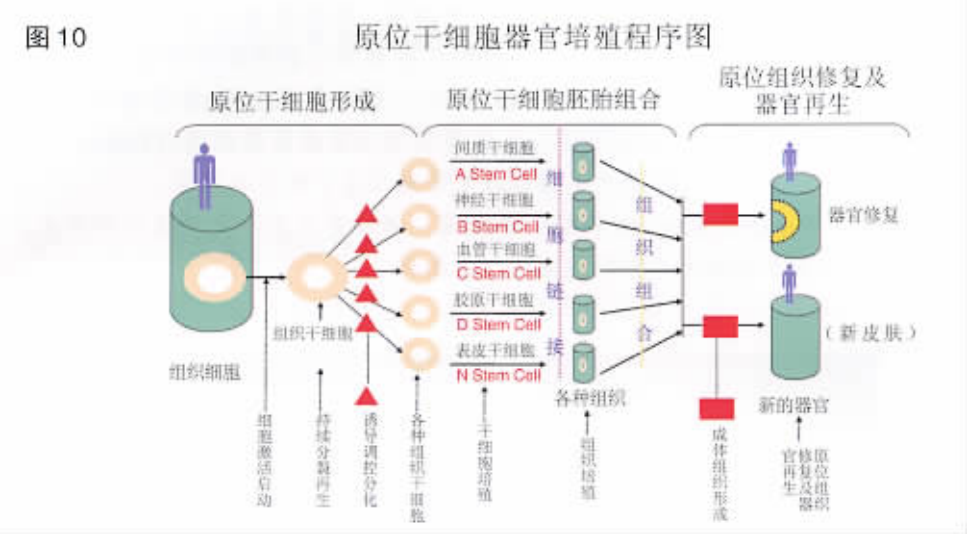
3．烧伤创面皮肤原位再生修复的过程：见图 10（原位潜能再生细胞再生复制皮肤器官程序 图谱）

从“原位潜能再生细胞再生复制皮肤器官程序图谱”中看出，皮肤再生医疗技术包括三个方面：一是原位激活启动潜能再生细胞，促进其形成干细胞；二是原位胚胎干细胞的组织组合；三是皮肤组织器官的再生组合。

1）原位激活启动潜能再生细胞形成干细胞：烧伤皮肤组织损伤后，产生大量炎性介质及有毒物质，并有大量细胞参与和许多因子释放，开始启动机体的生理性再生修复过程，激活存在于创面深层的潜能再生细胞形成干细胞。但这种激活的时间很短，仅在伤后 24 小时之内，若不能维持下去，便会转化为病理性修复过程，故需要外源性物质进行持续激活和维持，MEBO 可使干细胞持续性增殖，分裂再生。

万方数据

图 10



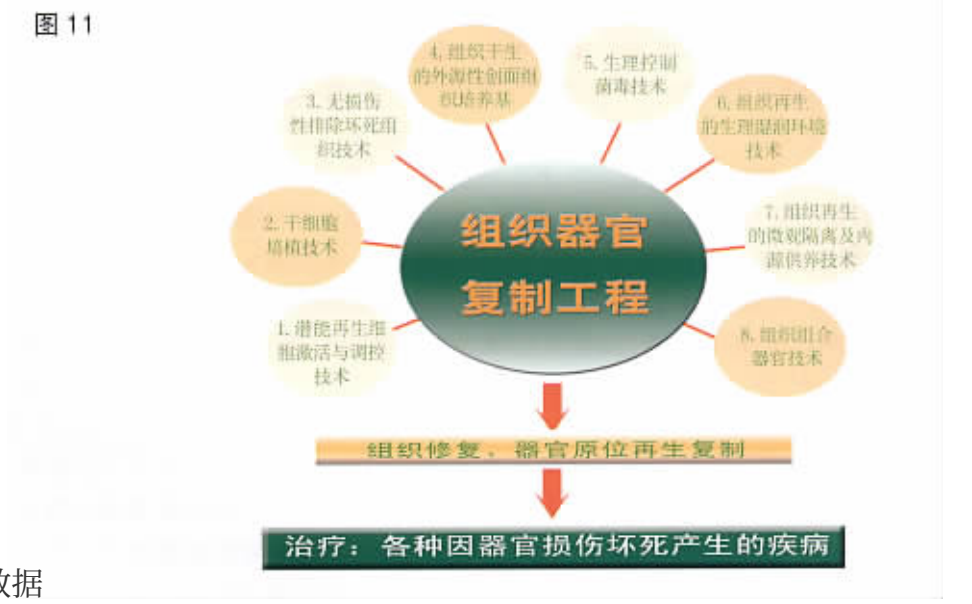
2）原位干细胞的组织组合：经诱导调控分化后形成的多能干细胞定向培植分化为多种专能干细胞（包括：表皮、胶原、纤维、血管、神经和间质干细胞等）。激活后的干细胞需要一种生命物质经常不断地保持其分裂、分化，成为表皮细胞、纤维细胞、神经细胞、血管细胞等，各种组织干细胞在 MEBO 所形成的生理性湿润环境中经过培植，细胞之间发生生理连接（桥粒连接），组合成正常的皮肤组织器官。

3）皮肤组织器官的再生组合：皮肤组织器官的生理性再生复制需要生理的调控机制，如成纤维细胞之间靠的是一种蛋白物质使之发生连接。根据皮肤细胞生态学特性，纤维细胞不能太多，否则将导致瘢痕增生，必须使上皮细胞和纤维细胞按 1∶4 的比例组合。表皮组织包括上皮细胞、基底细胞及基底膜等，真皮组织包括胶原、各种纤维组织、血管及神经等。成体皮肤组织器官形成后将与全身器官保持着紧密的生理性连接和神经体液的调节，从而完成了生理意义上的原位皮肤组织器官的复制。

三、烧伤创面皮肤原位再生修复技术的临床应用

根据创面原位再生修复的机制，可将烧伤皮肤再生疗法归纳为以下八项技术程序。这八项技术程序有严格的顺序和操作规范，是烧伤皮肤再生疗法能够产生最佳临床疗效的基础。见图 11（组织器官复制工程 八项技术程序图）

图 11



万方数据

烧伤皮肤再生疗法在临床应用中，有八项技术程序的调控参与实现残存皮肤组织修复和皮肤再生。用这个程序图不仅可以解释治愈各种原因引起的深度烧伤疗效的机制，同时也适用于许多皮肤粘膜疾病或人体多种组织器官损伤的再生修复。

1．激活受损伤皮肤组织深层的潜能再生细胞形成干细胞：这是皮肤再生疗法的起始阶段，但其效果与治疗方法密切相关。烧伤早期用药保护创面和深度创面的早期耕耘减张处理，可有利于激活潜能再生细胞和皮肤器官的原位再生。

2．原位培植干细胞：原位培植干细胞技术的关键是在创面上建立和保持 MEBO 形成的生理湿润环境，同时保护好创面上所形成的纤维隔离膜，因为皮肤组织原位再生过程均在这层膜的保护下完成。该膜的主要作用是以半透膜的代谢方式在创面和 MEBO 之间进行物质对流交换。所以在对创面的处置与治疗中必须格外注意纤维隔离膜的完整性，同时以持续的 MEBO 药物供给保持生理的湿润环境。

3．无损伤的液化排除坏死组织：严格按“三不原则”进行烧伤创面换药操作，为原位干细胞再生修复创造条件。

4．营养物质供应：干细胞分裂速度快，需要能量也大，体内本来具有适合于干细胞生长的物质，但在创面以胚胎发育形式再生时，这些营养成分显得十分不足，故需要内源和外源性供给再生修复所需的营养物质。MBEO 是仿生营养制剂，在 pH 和渗透压等指标都与人体内环境相似情况下，它具有较完备的创面再生所必需的营养物质，并与干细胞结合，故外源性的 MEBO 足以满足干细胞分裂增殖过程营养物质的所需。

5．采用生理性控制菌毒技术控制细菌和毒素的感染性伤害：原位干细胞培植技术对再生组织中微生物的处理原则与方法均不同于一般的无菌概念和抑菌技术，如果像一般的无菌概念那样，直接用抑菌杀细菌技术将会阻止干细胞的再生。所以，欲达到预防和控制原位干细胞培植技术中的微生物感染又不对再生细胞造成伤害，就必须采取不影响干细胞增殖的抑菌杀菌技术。MEBO 对细菌的干预为生理性调控机制，谓之非杀菌性调控，皮肤再生技术利用 MEBO 的功能，促使细菌变异和/或阻止、减少细菌毒性的技术。

6．保持组织的生理湿润环境：无论是创面局部还是胃肠道乃至全身，都需要生理性的湿润环境，烧伤创面只有在这种生理湿润环境中才能实现皮肤的原位再生修复，也包括全身脏器功能不全的调控与再生修复。

7．烧伤创面微观隔离技术和内源性供养技术：皮肤组织器官再生的微观隔离技术是创面活组织表面纤维隔离膜和 MEBO 药层共同实现的，只有建立创面再生的微生态环境，才能保障原位干细胞再生皮肤组织。

8．组织组合器官技术：在烧伤创面经过无损伤的液化排除坏死组织之后，最主要的是继续保持一个生理的湿润环境，无损伤的进行组织组合，形成以毛细血管树为核心，以纤维细胞为支架，使已经基本形成的皮肤干细胞依照皮肤的正常生理架构，组合成原始皮肤胚胎基和皮岛，此后还要继续用 MEBO 培养，使其真正达到皮肤组织器官全层的生理性再生痊愈。皮肤器官的组织组合是在人体的自身调节下完成的，而保障此过程的顺利进行必须创造良好的器官组织再生生理环境，没有生理的组织再生环境，所再生的组织不能组合成生理的皮肤器官。

【作者简介】

徐荣祥（1958－），男（汉族），山东沾化县人，1982 年毕业于青岛医学院，现从事再生医学研究，中国中西医结合学会烧伤专业委员会主任委员，《中国烧伤创疡杂志》主编，主任医师、教授。

萧摩（1943－），男（汉族），天津人，1965 年毕业于天津医学院，现从事烧伤皮肤再生医学研究，中国中西医结合学会烧伤专业委员会副主任委员、研究员。

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万方数据

The Mechanism of Burn Regenerative Therapy and Wound Healing

XU Rong – xiang, XIAO Mo

(China National Science and Technology Center for Burns, Wounds and Ulcers)

【**Abstract**】 **Objective**: From molecular, cell and systemic clinical treatment three levels, this report revealed the mechanism of wound healing, the basic theory and the importance of clinical practice for the Burn Regenerative Therapy with MEBT/MEBO (BRT & MEBT/MEBO). **Method**: This report analyzed the importance and clinical curative effect for standardized appliance of BRT & MEBT/MEBO from two key points: 1) Liquefaction of necrotic tissue from burn wound; 2) Skin regeneration *in situ*. **Result**: There are three necessary conditions for skin physiologically repair and regeneration of burn wound: 1) The formation of moist physiological environment on burn wound; 2) The material foundation of life regenerative substances and histology for keratin – 19 stem cells' regeneration *in situ*; 3) Standardized procedures and appliance of BRT & MEBT/MEBO, which is the guarantee of burn wound healing physiologically. **Conclusion**: The theory of "Potential Regenerative Cell" and the technique of "Stem cell *in situ* regeneration" are the basic theory and clinical treatment for BRT. Full – thickness burn skin can be healed physiologically and the skin tissues and organs can be regenerated ONLY by standardized procedures and timely appliance of BRT & MEBT/MEBO.

【**Key words**】: Burn; Burn Regenerative Therapy (BRT); Potential Regenerative Cell (PRC); Stem Cell *in situ*; Keratin-19 stem cell; In situ stem cell cultivation; fibrous isolation membrane; MEBT/MEBO.

INTRODUCTION:

Burn Regenerative Therapy (BRT) is based on the "Technique Core" of skin stem cell regeneration in situ and "Judgement Standard" of non – scar wound healing after deep burns. The main mechanism is setting up a moist physiological environment on the burn wound by liquefying the necrotic tissue without any further damage. Meanwhile, promoting the physiological wound healing by initiating PRCs and regenerating stem cells *in situ*. It's a very complicated physiological process for the liquefaction of the necrotic tissue and skin regeneration under the treatment of BRT & MEBT/MEBO. This report demonstrated the inevitability from BRT to wound healing physiologically in molecular, cell and systemic clinical treatment three levels.

In this technique, the liquefaction of the necrotic tissue is required to be done before the physiologically wound healing. If the skin necrotic tissues were excised followed by the traditional dry therapy, some of the interbiotic tissues (the tissues are alive and not necrotic completely yet) would be ineluctably excised too. More addition, lots of the "informative cells" with ectodermic gene massages and PRCs in wound basis will be cut too. These cells are the basic material foundation of wound repair and regeneration after burn, so the principle of clinical treatment of BRT is to protect these cells as much as possible, which will ensure the functions of transformation of stem cells and skin regeneration *in situ*. This is also the theory foundation of non – scar wound healing after deep burns under the treatment of BRT &

万方数据

MEBT/MEBO.

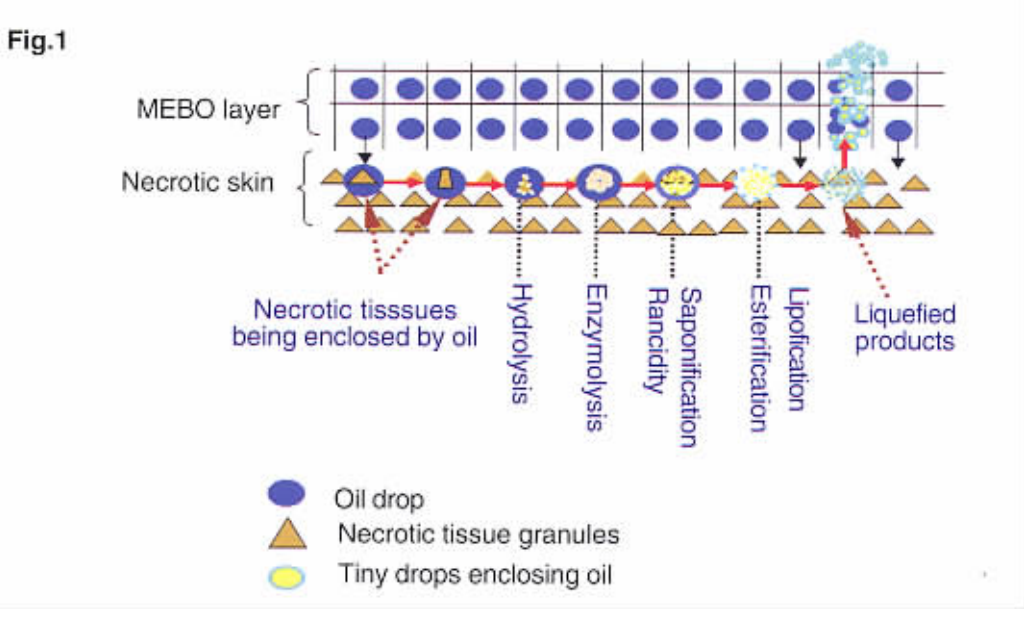
PART I: The practice of necrotic tissue liquefaction on burn wound

The liquefaction of the necrotic tissue on burn wound is a requirement for wound skin repair and regeneration *in situ*. The necrotic tissues on burn wound are liquefied and discharged without further damage must be applied under the strict standards and practice of MEBT/MEBO. The basic treatment principle is to reserve and protect the active tissue cells surviving in burn wound basis, so that the interbiotic tissues can be resuscitated under the moist physiological environment, which ensures the wound repair and regeneration.

(i) The process of necrotic tissue liquefaction:

The solid necrotic tissues can be changed to liquid form (Liquefaction), and then discharged from the wound under the effect of MEBO. Before we annotate the process of liquefaction, we need to introduce the pharmacological effect of the special frame form of MEBO (low melting point). This frame form is alterable depend on the change of wound temperature: MEBO is an ointment at Room Temperature; after applied onto the wound for the thickness of 1mm, there are two layers of MEBO: the outer side of MEBO keeps "ointment form"; while the wound – touched side is wormed up and transformed to liquid form. MEBO has the lipophilic character. After applying, the liquid form MEBO reacts with necrotic tissues on burn wound, and then MEBO loses the lipophilic character and mixes with exudation and liquefactive stuff, then the mixture moves to the outer layer of MEBO and be discharged out of skin. The new upper layer of MEBO continues to be warmed up and transformed to liquid form and then Hydrolysis, Enzymolysis, Rancidity, Saponification and Esterification happen sequentially, repeatedly and drainage automatically. This is the main mechanism of liquefaction of burn wound necrotic tissue (See Figure 1).

Fig.1



A. MEBO is warmed up and surround necrotic tissues: First, the grease base of solid form MEBO is warmed up by wound temperature and be transformed to liquid form, the oil is released and flows into the burn wound, divides the necrotic tissue into pieces and surrounds them, which will initiate the series chemical reactions between MEBO and necrotic tissues. (See Figure 2)

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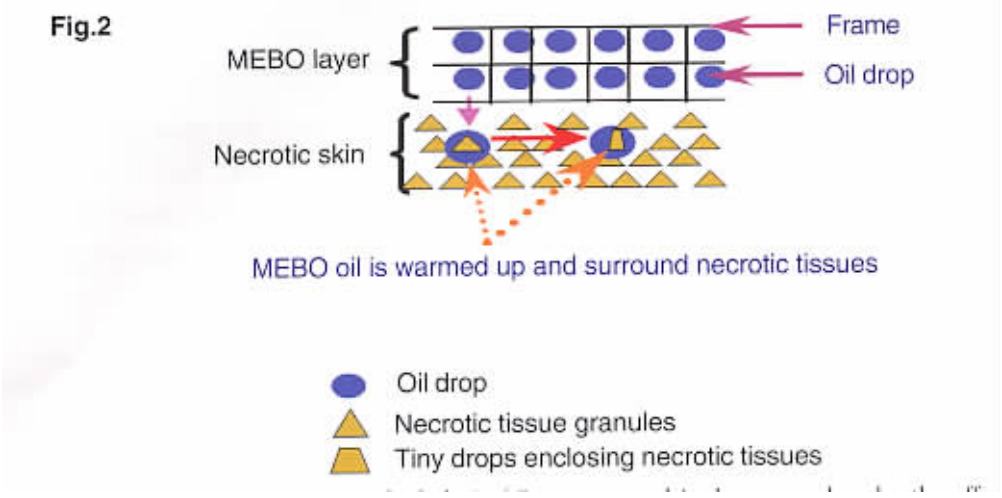
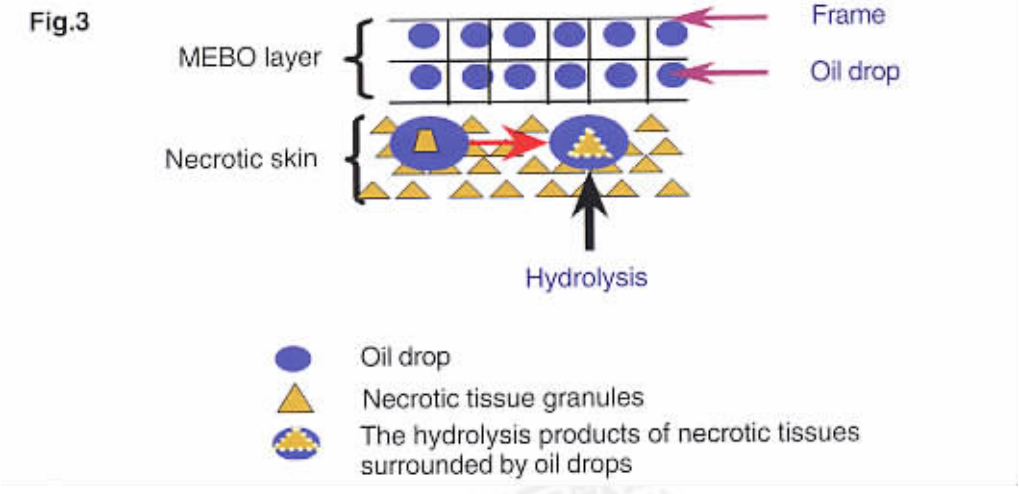
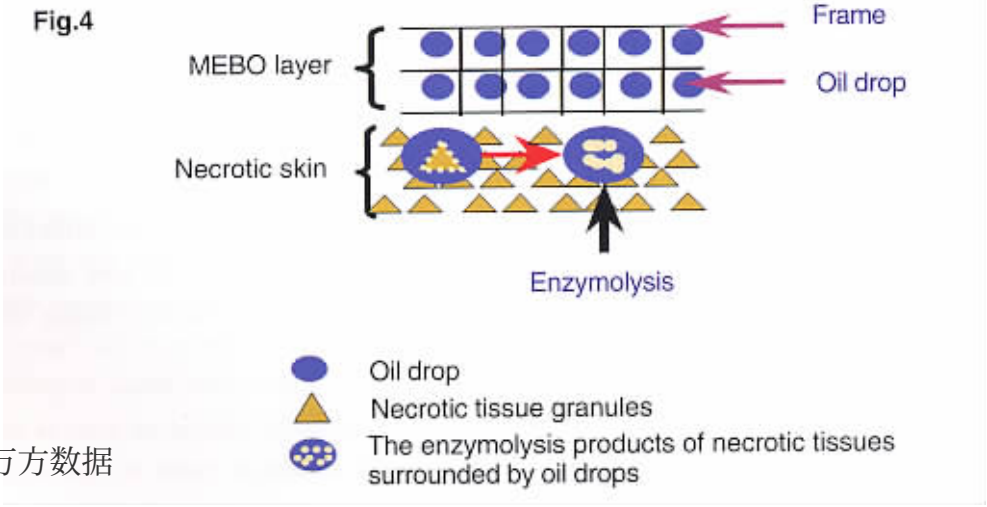


B. Hydrolysis: The first reaction is hydrolysis (One compound is decomposed under the effect of water.) The remain water in necrotic tissues react with the necrotic skin under the effect and surrounding of MEBO, which will further initiate the series chemical reactions. (See Figure 3)



C. Enzymolysis: There are kinds of zymngens in remnant cells in burn wound necrotic tissues. Zymngens are the non – activate prosome of enzymes. After hydrolysis, the peptide of the zymngen in charge of the non – active function is removed. Then the big molecules such as protein, fat, carbohydrate are digested into small molecules by several kinds of enzymes. Thus, the necrotic tissue on burn wound is changed into molecular level. (See Figure 4)



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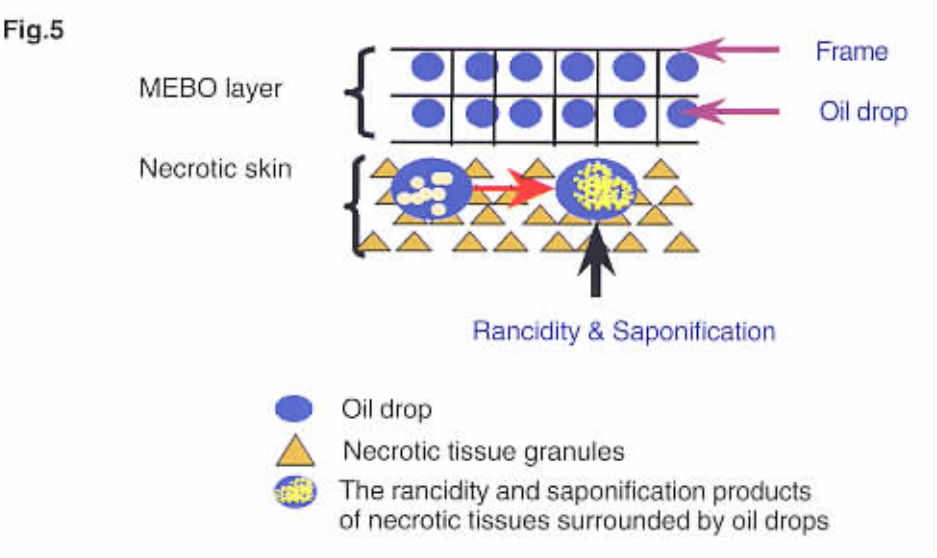
D. Rancidity and saponification: Based on organic chemistry, acid and alkali chemical reactions are the reaction that obtain or lose electron (s). Rancidity reaction means that the amino acids, fatty acids are separated, the aldehyde keton oxide are formed from protein, fat tissues after the above – mentioned reactions. All these acidity compound are composed by hydrogen ion and acid radical. These organic acids produce neutral salt and water after decomposion and combination. Saponification means the fat hydrolyzes in the alkalescence solution and produces glycerol and fatty acids.

Burns can make the wound tissue denaturalized and necrosis, produce lots of histamine, bradykinin, lactic acid, free radical and other acid substances, which are called" Burn toxin ". Under the therapeutical effect of MEBO, the burn toxins are decomposed, so that the direct damage of the wound are relieved; more addition, it reduces the damages of multi-organs in the body by absorption of burn toxins. In one word, the result of rancidity and saponification is to decompose the necrotic tissue into neutral substances, which will protect the wound efficiently and alleviate the damage of burn toxins after absorption. (See Figure 5)

Fig.5



E. Lipofication and Esterification: The necrotic tissues are transformed into liquid form after above – mentioned reactions. The liquid necrotic tissues then react with surrounding MEBO and perform lipofication and esterification, which will ensure the necrotic tissue be liquefied and discharged from the wound eventually. MEBO has net – like frame form. It ' s composed by grease and surrounding beeswax. There are abundant linoleic acids (belongs to non – saturation acid), which can be decomposed with sterol stuff, aldehyde oxide, keton oxide, lipid, adipoidand, etc. This process is called" lipofication ". After that, the linoleic acids in MEBO bind with those substances and form esters, this is" esterification ".

In the process of liquefaction, esters can be degraded into acids, the opposite reaction is the acids are transformed into esters under the effect of esterases. The acid burn toxicity in necrotic tissue is reduced through these reactions, which also protect the moist physiological environment of burn wound and prevent its further damage. More addition, esters compound can protect surviving tissue of burn wound, promote wound healing and cultivate the neo-regenerated skin tissue. Thus, it can be seen that Lipofication and Esterification are reversible biochemical reactions happening one after another. In this step, the decomposition between grease (linoleic acids) and liquefactant of necrotic tissue is not only the main base of burn wound moist physiological environment, but also the main elements which promote decomposition and liquefaction. After that, the necrotic tissue are transformed into liquid form of esters liquid mixture, the toxicity and irritation are much reduced and the mixture are easily to be discharged, which ensures the process of skin organ regeneration *in situ*. (See Figure 6)

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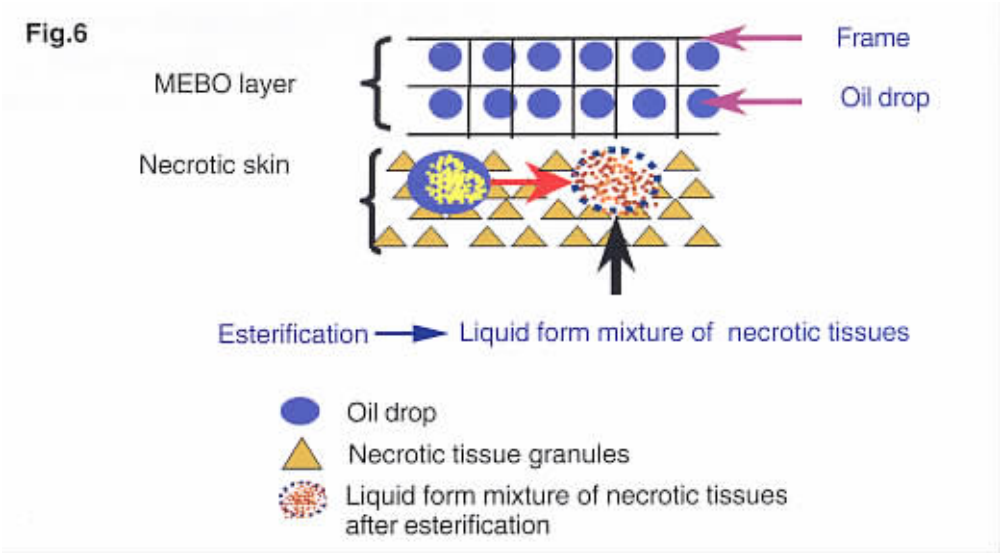
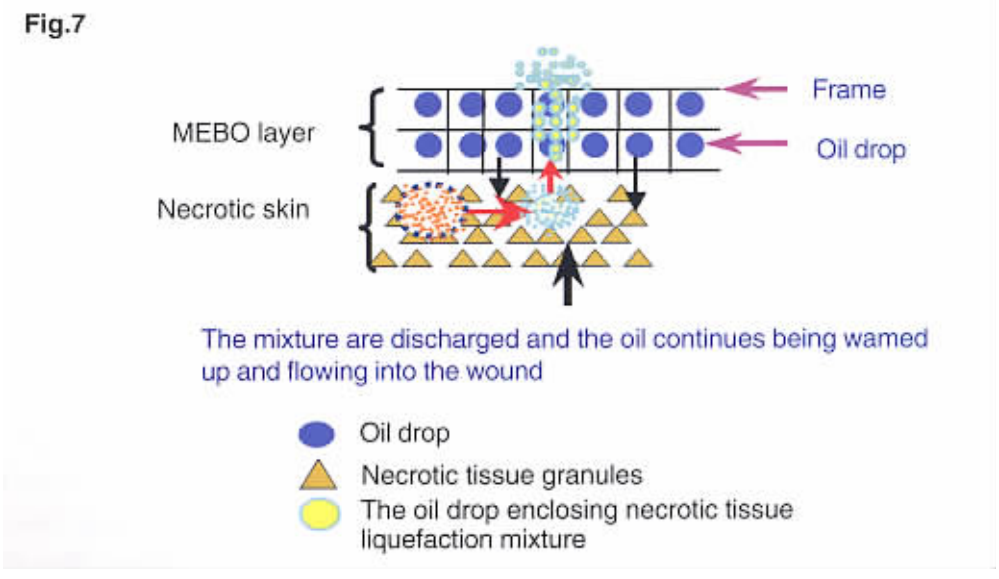


The biochemical reactions of MEBO occurred in the burn wounds enable necrotic skin tissue particles to become liquefied mixtures, so that necrotic skin tissues can be removed without causing any further damages through local application of the drug according to MEBT, to ensure regenerative repair of the wound. All this ensures the physiological wound healing without scar. To be emphasized, in order to fulfill the clinical effect of BRT & MEBT/MEBO, not only the correct and standardized application of BRT is a requirement in local wound, but also the procedures and techniques of systemic BRT, they assistant to each other. (See Figure 7)



(ii) The practice methods in liquefaction period:

After removal of liquefied necrotic tissues, a fibrous isolation membrane is formed on the wounds surface like a layer of transparent cornea. It is very important to protect this membrane (to be explained later).

In clinical practices, wound necrotic tissue liquefaction should be distinguished from wound infection. Infection is a process that inflammation reaction is taking place in viable tissues affected by pathogenic bacteria. Infection always brings on the purulence and necrosis of living tissues. Infection is always characterized by local redness, swelling, fever, pain and dysfunction. While, liquefaction is a process that necrotic tissue under the action of MEBO is turned automatically

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from solid state to liquid state and be removed without causing any further damage. There is no living tissue involved in this process. A layer of milky white or milky yellow, homogeneous, fine and smooth semi – fluid substance is formed on the wounds and is removed through changing of the dressings without causing any damage. The following principles should be followed:

A. Early application of the drug: It is recommended to apply the drug within 4 hrs post injury. The purpose is to save the tissues in the stasis zone of the wounds and prevent progressive necrosis to ensure the efficacy of the treatment.

B. The drug should be applied in the whole course of the treatment without interruption: No other drug or treatment should be used to replace it in the whole course of treatment. Dry therapy and astringent drugs are forbidden. Antiseptic agent is not allowed to apply directly on the wounds. Don't use water to wash the wounds.

C. Wound dressing change should be carried out in a standardized procedure: Liquefied products should be removed every 4 hrs. Handle carefully, don't cause pain, don't cause bleeding and don't cause any further damage in the normal skin tissue. It is very important to protect the fibrous isolation membrane to cover the wound. Adhere to the "Three DON'T principle" so that physiological regenerative repair of the wound can be achieved.

D. Wound treatment: "Three timely principle" is one important principle in the management of wounds, i. e. timely removal of liquefied products, timely removal of necrotic tissue and timely application of the drug. Another key principle is "Three no accumulation", i. e. no accumulation of necrotic tissue, no accumulation of liquefied products and no accumulation of excessive drug (MEBO).

In the treatment of large area burn wounds, it is very important to strictly follow the above principles not only to ensure quick healing of the wounds, but also directly related to the systemic condition and recovery of the patients. This can help reduce the amount of bacteria and the absorption of inflammatory and burn toxins produced in the necrotic tissue and is the key to effective improvement of the systemic condition and lessening of the symptom of intoxication.

PART II: The mechanism of regenerative repair of burn wounds *in situ*

There are three requirements for skin regenerative repair of burn wounds: First of all, a moist physiological environment should be formed in the wound; secondly, there should have histological basis for *in situ* cultivation of skin keratin type 19 stem cell and material basis for regeneration; and thirdly, standardized therapeutic technique of skin regeneration medicine should be applied, in order to ensure skin regenerative repair of burn wounds.

(i) The formation of a moist physiological environment on burn wounds:

In course of the application of BRT & MEBT/MEBO to promote liquefaction and removal of necrotic tissue in burn wound, a moist physiological environment is formed simultaneously. After discharge and liquefy of necrotic tissue, a layer of transparent, cornea – like fibrous isolation membrane is formed on the wound surface. The presence of this membrane is a sign of the formation of moist physiological environment. This membrane protects the viability of the skin tissue organs regenerated from stem cells *in situ*, so that it is important to protect this membrane in order to ensure physiological regenerative repair of the burn wounds.

Experiments proved that when the wounds were protected by MEBO + fibrous isolation membrane, the evaporation of the burn wound tissue is approximately the same as that of the normal skin. This membrane protects the injured wounds and helps wound respiration. This is quite different from dry therapy and vaseline treatment, the burn wounds do not lose a lot of water as treated with dry therapy and the tissues are not suffocated and macerated as treated with vaseline.

A. The formation of fibrous isolation membrane: MEBO has good compatibility with burn wound exudates and can form a fibrous isolation membrane in the course of wound necrotic tissue liquefaction. The membrane is composed of lipoids produced by the biochemical reactions of MEBO and lipoproteins complexes produced by exudates of plasma protein from wounds. The membrane tightly covers the wound surface, so that the neo-regenerative stuff can attached to it and the wounds can be repaired in a regenerative way in moist physiological environment.

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B. The characteristics and function of the fibrous isolation membrane: As a "semi – permeable" membrane, the special composition of the fibrous isolation membrane enables its unique characteristics and function. It lies in between wound surface and MEBO. In both sides of the membrane, osmotic pressure is formed respectively. One side is hydrophilic (wound tissue) and the other side is lipophilic (MEBO). Exchanges of materials as ions are taking place, so that the metabolism products of the wounds can be excreted into the outer side of the membrane. Through the same mechanism, the nutritive and biologically active components of MEBO can go into the deep layer of the wounds and to give full play of its physiological and pharmacological effects to promote skin tissue regeneration and repair. (See Figure 8)

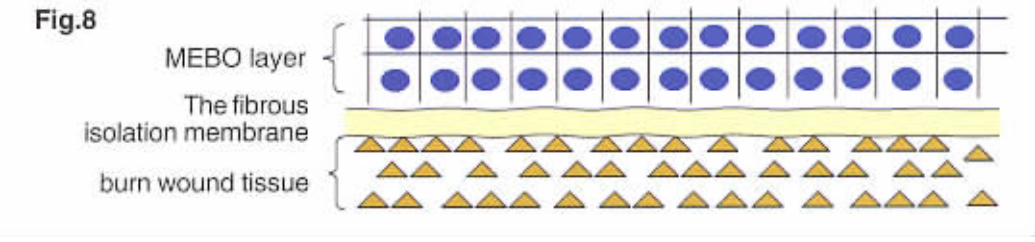

Fig 8. An illustrative sketch of fibrous isolation membrane

C. Clinical significance of fibrous isolation membrane: The whole process of burn wound regenerative repair in situ is completed by the protection of the fibrous isolation membrane under the standardized application of BRT & MEBT/MEBO. Only under the protection of the isolation membrane could the microcirculation in the deep layer of the wound be regenerated in the frame of physiological capillary tree, so that nutritive materials could be transferred to the primitive skin blastemata and skin islands, which were regenerated in situ by stem cells and developed and expanded to heal the wounds. Serious attention should be taken during change of dressings, washing and cleaning, to keep the fibrous isolation membrane intact. The wounds should be kept in physiological moist state, not dried nor macerated, through continuous supply of the drug. That's why the principle of "three no injury" and "three no accumulation" in wound management was suggested.

(ii) In situ cultivation of skin stem cells:

The discovery of Potential Regenerative Cells (PRCs) and the application of the technique of in situ stem cell cultivation for skin regenerative therapy are the histological bases of burn wound skin regeneration in situ. The PRCs for in situ regeneration of skin organs are the prosome of the skin keratin type 19 stem cells, which are differentiated from cells in subcutaneous tissue. Burn wounds deep to skeleton can be regenerated and repaired by introducing bone marrow cells into subcutaneous tissue through drilling holes on the bone in situ, then the PRCs are differentiated into keratin type 19 stem cells to achieve regenerative repair of the wounds in situ.

In the course of metabolism of healthy tissues and organs, the processes of apoptosis, degeneration, injury and necrosis are going unceasingly and at the same time replenishment of new born cells are going on as well, to maintain the structure and physiological function of the tissue organs. The process of the recovery of the lost local tissues is called regeneration. Physiological regeneration is a normal process of replacing the old cells by new ones. Regeneration is an instinct of human beings. Through 10 years' research, we primarily resolve this riddle and explain the law of the life continuous, the research results were released in 2002. PRCs are the origin of in situ stem cell cultivation regeneration and repair technique on burn wound. The reveal of this life law gives a scientific demonstration to the physiological regeneration of burn wound in BRT.

A. The definition of Potential Regenerative Cell (PRCs): PRCs are the special differentiated tissue cells, which have the potential ability to regenerate to a functional tissue similar as stem cells but normally exist in tissue as static tissue cells. (See Figure 9)

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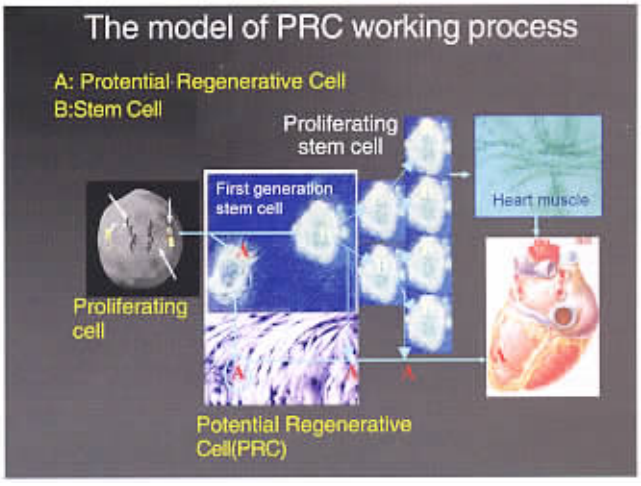
Fig.9



The division of cells with the appearance of spindle fibers is called mitosis. During mitosis, chromosomes duplicate and divide themselves equally into two daughter cells. This is "Symmetry Splitting". While in the process of cell split, some cells perform "Asymmetry Splitting", in which, some of the new cells continue to divide and some of them stop dividing, and become tissue cells directly, which are the main source of PRCs.

PRCs in the tissue organs are produced by original and multi – potential stem cells in different periods of organ development. Generally, these PRCs exist as common cells, but when apoptosis, degeneration, injury and necrosis of some cells in the tissue organs take place, these PRCs will proliferate to produce new cells, in order to fill in the vacancy resulted from loss of cells and to maintain the structure and the function of the organs. This regeneration activity helps maintain the balance of human life.

B. The concept of burn wound regenerative repair *in situ*: The concept of burn wound regenerative repair using skin regeneration medical technique (BRT) is that: The wounds are placed in a moist physiological environment and life regenerative substances (MEBO) are supplied continuously to the cells, so that PRCs are initiated in situ to form stem cells and then proliferate, regenerate, differentiate into different types of tissue stem cells. Adult tissue is formed through cultivation, connection and combination of different tissue stem cells. The regenerative repair of burn wound in situ is completed eventually.

C. The course of burn wound regenerative repair *in situ*: (See Figure 10)

Fig.10

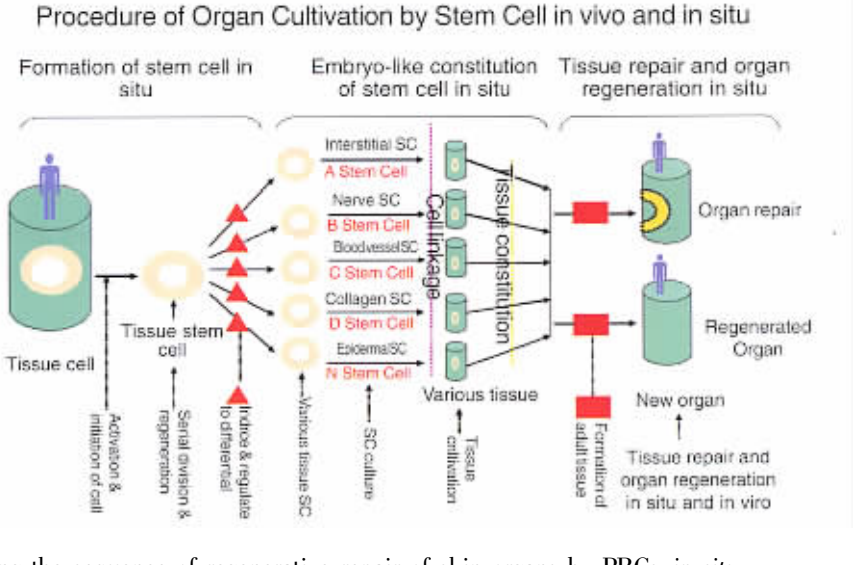


Fig. 10. Atlas showing the sequence of regenerative repair of skin organs by PRCs *in situ*

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From figure 10, we can see that the technique of skin regenerative medicine comprises 3 parts: First of all, the PRCs are initiated and activated to form adult stem cells in situ; secondly, the adult stem cells in situ are differentiated and form different tissues; and finally, the skin organ is regenerated after the combination of all types of neo – burn tissue organs.

a) The initiation and activation of PRCs in situ to form stem cells: After the skin tissues are injured thermally, a lot of poisonous inflammatory media are produced. A great amount of cells are involved and active factors are released, so that the process of physiological regenerative repair is started and PRCs in the deep layer of the wounds are initiated and activated to form stem cells. But the duration of activation is very short, within 24 hrs post burn, if this process could not continue, pathological repair process would take place instead of physiological regeneration. That's why exogenous materials are necessary to continue the activation of PRCs. MEBO has the ability of maintaining continuous proliferation and regeneration of PRCs and the stem cells.

b) Combination of stem cells in situ to form tissues: The adult stem cells formed after induction, regulation and differentiation are continuously cultivated in MEBO to form different directional specific stem cells including epidermis, collagen, fiber, blood vessel, nerve and mesenchymal stem cells. The activated stem cells need life regenerative substances to maintain their activities to proliferate and differentiate to form tissue cells, such as epidermal cells, fibrocytes, nerve cells, vascular cells, etc. This process is performed in the moist physiological environment created by MEBO. Intercellular physiological connections (such as desmosome connection) of these cells result in the formation of normal skin tissue organs.

c) Regenerative combination of skin tissue organs: Epidermal tissues include epithelial cells and basement membranes. Dermal tissues include collagen, fibrous tissues, blood vessels and nerves, etc. Skin tissue organs' physiological regeneration and repair needs physiological regulation system. For example, the presence of a kind of protein is needed for the connection between fibroblats. According to the ecology of skin cells, there should be no excessive fibrocytes, otherwise, hyperplastic scar will form. The ratio of the combination between epithelial cell and fibrocyte should be 1:4. After the formation of skin tissue organs, the organs as parts of the body are closely connected to the whole body through physiological and neurohumoral regulation. Thus, the process of regeneration and replication of skin tissue organ in situ is completed.

PART III: Clinical application of regenerative repair of burn wounds in situ

According to the mechanism of in situ regenerative repair of wounds, burn regenerative therapy can be described as 8 technical procedures. These 8 procedures should be followed in strict and standardized sequence and application. This is the foundation that BRT & MEBT/MEBO can achieve best clinical results. (See Figure 11)

Burn regeneration therapy, under the control of the 8 procedures, can achieve surviving residual skin tissue repair and skin regeneration. Fig. 11 not only explains the mechanism of the treatment of deep burn wounds of various causes, but also is applicable to the regenerative repair of various skin mucous membrane lesions and tissue organ injuries.

(i) First of all, the PRCs in the deep layer of the injured skin tissue are activated to form stem cells:

This is the start period of BRT. The effect has a close correction with the treatment method. Early application of the drug to protect the wound surface and "Ploughing method" to reduce the tension of deep wounds can help to activate of PRCs and skin organ regeneration in situ.

(ii) In situ cultivation of stem cells:

The key point for this procedure is to maintain a moist physiological environment created by MEBO and also protect the fibrous isolation membrane formed on the wound surface. This semi – permeable membrane allows the exchange of materials between both sides, from wound to MEBO.

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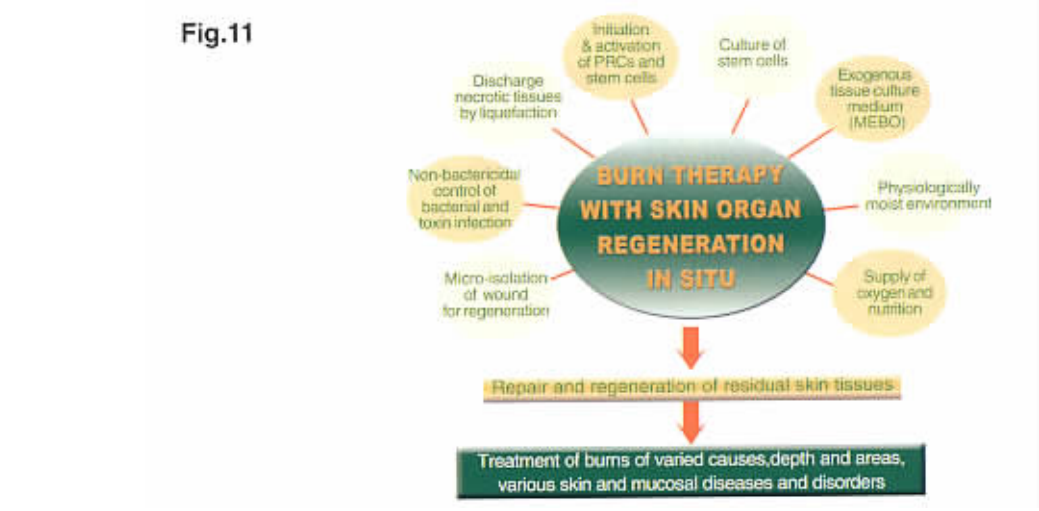

Fig 11 Tissue organ duplication project 8 technical procedures

(iii) Necrotic tissue should be liquefied and removed without any further damage, to create a condition for stem cell regeneration and repair *in situ*.

(iv) Nutrition supply:

Stem cells proliferate very quickly and consume a lot of energy. Although there are enough nutritive materials for stem cells' growth in the body, when stem cells regenerate in an embryo development model, nutritive materials become very insufficient. Exogenous and endogenous supply of the nutritive materials and physiological regulation are necessary. MEBO is a bionic nutritional preparation, its pH value and osmotic pressure are similar to that in the human body, it can provide all the necessary regenerative substances and nutrition for wound healing and regeneration and can bind stem cells, so continuous supply of MEBO will satisfy the nutrition supply of stem cell proliferation process.

(v) Using physiological controlling technique to control bacterial and toxic infectious injuries is a non – bactericidal controlling technique:

In the in situ stem cell cultivation technique, the treatment principle and method for microorganism in regenerative tissues is different with normal conception of sterility and technique of anti-bacteria. Stem cell and tissue regeneration is inhibited by antibiotics which are used in large doses in dry therapy. Thus, in order to prevent the damage of microorganism infection to regenerative cells, a non-bactericidal controlling technique must be taken. MEBO can control bacterial infection and at the same time promote immunity of human body, so it has a comprehensive anti – infection effect.

(vi) To maintain a moist physiological environment:

All the wounds regardless of the location, a local wound or wounds all over the body, or wound in the gastrointestinal tract, need a moist physiological environment. Only in such an environment could skin regenerative repair be realized. This also includes the regulation and repair of dysfunction of the organs all over the body.

(vii) Micro – isolation of burn wound and endogenous nutrition supply technique:

The micro – isolation technique for skin tissue organ regeneration is realized by the" fibrous isolation membrane "together with" MEBO drug layer". Only after setting up the micro – ecosystem, could the skin regeneration from stem cell *in situ* be fulfilled.

(viii) Tissue combination to form organs:

After liquefaction and removal of necrotic tissue, wounds should be kept in a moist physiological environment. Tissues are combined without causing any damage, to form a capillary tree core and a fibrocyte trestle, so that skin stem cells can combine according to the normal physiological structure of the skin, to form primitive skin blastema and skin island. MEBO should be supplied continuously to achieve full – thickness, physiological and regenerative repair of the skin tissue organs.

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translated by SUN Xia, LIU Zheng – gu