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E2f1 Represses M1 And M2 Macrophages Transformation To Effect Wound Healing Process

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PURPOSE: Wound healing is a complex process, which is classically divided into inflammation, proliferation and remodeling phases. Macrophages play a key role in wound healing. M1 macrophages mediate tissue damage and initiate the inflammatory response in the early stages of wound healing. M2 phenotype promotes wound healing via formation of a highly vascularized, cellular granulation tissue and scar tissues. The phenotype of polarized M1-M2 macrophage can, to some extent, be reversed in vitro and in vivo. It is not clear whether the mechanism of this switch involves the recruitment of circulating precursors or the reeducation of cells in situ. In our previous study, we found that E2F1-null (E2F1−/−) mice have enhanced expression of macrophages in the border zone of the skin wound at day 7 post-surgery. However, whether E2F1 mediates the M1-M2 switch during the wound healing process is not known.

METHODS: Skin wounds were surgically induced in E2F1−/− mice and the WT littermate. At 2th and 7th day after surgery, we detected the numbers of M1 and M2 macrophages in the border zone of the wound. Then we performed Western-blotting and RT-PCR to investigate the PPAR-γ protein and RNA expression in the wound tissue. And Co-IP was performed to check whether E2F1 interaction with PPAR-γ.

RESULTS: In the border zone of the wound, E2F1−/− mice had more M2 macrophages and less M1 macrophages at day 7 post-surgery. Surprisingly, at day 2, the M2 macrophages were also remarkably increased in the E2F1−/− mice, which suggests a certain degree of transformation amongst the M1 and M2 phenotypes on the 2nd day. We know that PPAR-γ plays a key role in the M1-M2 switch. However, whether E2F1 interacts with PPAR-γ during the wound healing process is not known. We performed Co-IP and found that E2F1 indeed interacts with PPAR-γ. Western-blotting and RT-PCR showed higher expression of PPAR-γ in the E2F1−/− mice as compared to that in the WT mice.

CONCLUSION: E2F1 may repress PPAR-γ expression to affect M1-M2 macrophage switch that prevents skin wound healing.

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Micropatterned Microsphere Scaffolds: Optimizing the Performance of Engineered Dermal Substitutes

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PURPOSE: Current dermal replacement products perform sub-optimally in complex wound beds, such as those that have been irradiated or those with exposed hardware, mostly as a result of insufficient cell invasion and vascularization. We have previously observed more robust endothelial cell invasion of scaffolds containing a micropatterned matrix of differential collagen stiffness in a murine model when compared to collagen controls. Herein we compare the performance of our micropatterned microsphere hydrogels (MSS) to a widely utilized commercially available dermal substitute. This enhanced cellular invasion and accelerated neovascularization, which results solely from the unique architecture of the scaffolds, indicates superior efficacy in the rate of integration into the host wound bed and may result in decreased length of time between its application and definitive wound closure. Further, the significantly more robust cellular and vascular invasion and integration of these scaffolds indicates their applicability in the treatment of suboptimal wound beds, which is beyond the capability of currently available dermal substitutes.

METHODS: Microspheres composed of 1% type I collagen 50-150um in diameter were created and encased in a 0.3% type I collagen bulk. For our in vitro study, polydimethylsiloxane (PDMS) wells of 4mm diameter and 2mm height were filled with the microsphere scaffolds. 3x2mm Integra® disks were placed inside PDMS wells. Non-microsphere containing 1% and 0.3% collagen scaffolds served as controls. A monolayer of endothelial cells was seeded onto this three-dimensional platform, activated for invasion with 1uM sphingosine-1-phosphate, and cultured for 3 days. The collagen hydrogels were then analyzed using confocal microscopy to quantify cell invasion. For our in vivo study, 8x2mm MSS disks were created, along with 1% and 0.3% collagen controls. 8mm Integra® disks were created, and the unilateral silicone layer was removed.