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Wound Healing Revealed by a Novel Automated Indentation Technique

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Problem: Mechanical characterization of wound healing in skin samples mostly relies on uniaxial tensile rupture tests providing local information along the wound and are disruptive for samples.

Objectives: In this study, we wanted to test the ability of a novel automated indentation technique to non-destructively characterize mechanical properties of the entire wound and its integration with the surrounding skin.

Methodology: Wounded pig skin samples were placed skin surface up on a platform of a multiaxial mechanical tester (Mach-1v500css, Biomomentum Inc., Canada). Following top-view photodocumentation, a position grid (>130 positions) was superimposed over the image. At each position, the tester was programmed to precisely measure skin thickness and to perform an indentation ramp of 1.5 mm at 200 µm/s with a spherical indenter (6.35 mm diameter). Subsequently, the sample was reshaped in two adjacent dumbbell-shaped strips (perpendicularly to the wound), mounted in tension grips and tensile rupture tests were performed at 2 mm/s.

Results: High-resolution mapping of maximum load and thickness were generated (about 30 s per position). These mappings revealed significant spatial variation of the mechanical properties and thickness over the wound region compared to the uniform properties of the intact skin observed at least 1 cm away from the incision site. Considering the load at rupture in the tension tests, a correlation could be observed with the maximum load in indentation (or thickness) measured at the rupture site.

Significance: These results indicate that this indentation technique can provide a novel assessment of mechanical properties revealing the 2-dimensional distribution over the wound and its surrounding areas.

Donor Factors and Stem Cell Type Affect the Potential and Multipotency of Human Adipose Tissue-Derived Stem Cells

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Objectives: Human adipose tissue-derived mesenchymal stem cells (AT-MSCs) have different characteristics depending on donor characteristics. AT-MSCs also differs from MSCs of other origins. This study investigated the differentiation and proliferation characteristics depending on age, body mass index (BMI), gender, and origin of MSC.

Methodology: AT-MSCs from 66 human donors were sorted according to donor age (10–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70 years and older), BMI (under 25 kg/m^2, 25–30 kg/m^2, and over 30 kg/m^2), and gender. AT-MSCs were also compared with bone marrow MSCs and chorionic tissue-derived MSCs. MSC yield, growth rate, colony-forming units, differentiation potency (ELISA analysis after Oil Red O staining for adipogenic, Alizarin Red S staining for osteogenic, and Alcian Blue staining for chondrogenic differentiation), and surface antigens(CD90, 13, HLA-DR, CD105, CD34, CD73, CD45, CD146) were compared.

Results: AT-MSC proliferation was greater in cells isolated from donors aged less than 30 years compared to cells from donors over 50 years old. Adipogenic differentiation was more strongly induced in cells isolated from donors less than 30 years compared to other age groups. BMI above 30 was associated with enhanced adipogenic differentiation compared to cells isolated from individuals with a BMI lower than 25. Cells from donors with higher BMI had better osteogenic differentiation potency. Bone marrow MSCs sh1)owed stronger osteogenic and adipogenic lineage differentiation, while AT-MSCs predominantly differentiated into the chondrogenic lineage.

Conclusions: The type of regeneration required, and variations among donors and type should be carefully contemplated when selecting MSCs to apply in tissue engineering or cell therapy.

A Novel Approach to Study The Immunoregulatory Effect of Exogenous Factors on Macrophages

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Macrophages, acting as both immune cells and osteoclast precursors, play an important role in bone homeostasis. The traditional approaches to study macrophages are based on the stimulation of pathogens which can be rarely found under homeostatic conditions. This study aims to create a novel approach to investigate the immunoloregulatory effect of exogenous factors on macrophages under both physiological and pathological conditions. A murine macrophage cell line RAW264.7 was cultured in normal growth medium supplemented with different concentrations of the exogenous vascular endothelial growth factor (VEGF). Our results showed that the expressions of inflammatory cytokines in RAW264.7 cells enhanced consistently with the increasing concentration of VEGF with or without lipopolysaccharide (LPS) preconditioning. The same results were found when the VEGF was replaced with the conditioned medium from the osteogenically differentiated bone marrow-derived mesenchymal stromal cells (BMSCs) without the presence of LPS. The present study demonstrated that the immunoregulatory effect of exogenous factors on macrophages could be observed without pathogenic stimuli. The established method could better reflect the *in vivo* bone remodelling process when no inflammation is involved. It is a feasible approach to study the interaction between the target protein and the immune cells under non-inflammatory conditions.

Use of Skin Substitutes in a Burn Animal Model

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Available treatments in skin regeneration are insufficient to promote healing. This study has aimed to produce a cutaneous substitute joining mesenchymal stem cells (MSCs), keratinocytes and a PDLLA biomaterial constructed by the electrospinning technique for use in nude mice. Five groups were tested: (1) PDLLA without cells; (2) PDLLA/Lam, hydrolyzed scaffold with laminin binding, without cells; (3) PDLLA with cells; (4) PDLLA/Lam with cells (n=6/group) and (5) animals injured without scaffolds (control), in which gauze was used (n=4). All the animals had 1 cm2 cutaneous defect performed on their backs, removing all the skin. The scaffolds were implanted in the mice with imitation burn skin defects for up to 9 days. Photographs were taken on the days of surgery and euthanasia. Part of the skin defect was used for histology analysis and another part for gene expression evaluation. After 9 days, the cutaneous defect size for group 1 was 0.439 ± 0.008 cm^2 and 0.315 ± 0.003 cm^2 for group 2. The scaffolds in which the cells were seeded presented similar results, with a defect size for group 3 and 4 of 0.411 ± 0.017 cm^2 and 0.342 ± 0.021 cm^2, respectively. The control group showed a 0.319 ± 0.003 cm^2 defect size. Group 2 presented the best appearance in the lesion, with the softest wound. The histological and gene expression analysis are currently being performed. However, it was clear that the scaffolds, mainly PDLLA/Lam, improved the quality of the healing process of the wound in this animal model.

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