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Human breast milk exosomes accelerate mouse wound healing

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The healing of cutaneous wounds is a very efficient process despite being very complex. Under certain pathological conditions healing may be impaired, prolonging the healing process and eventually leading to medical intervention and the chronicity of the wound. Exosomes are secreted extracellular vesicles present in biological fluids where they play a key role in intercellular communication at the tissue, organ and organismal levels. Given that human breast milk contains abundant maternal extracellular vesicles (MEVs) with pro-regenerative and immunomodulatory properties, the aim of the present study was to evaluate if topical application of MEVs into open wounds would be beneficial for healing. Full-thickness excision wounds of 4-mm diameter were created in the dorsal skin of C57BL/6 mice and topical application of 20 micrograms of human MEV isolated at weeks 9, 11, 12 and 15 postpartum of breastfeeding were placed onto the open wounds. Control wounds were vehicle-treated. Macroscopic measurements up to 7 days post-wounding revealed that the area of the wound treated with MEV significantly decreased compared with controls. Histological analyses at day 7 post-wounding showed no differences in the granulation tissue area between groups. Nevertheless, dermal integrity of the wounds appeared to vary under MEV treatments isolated at different breastfeeding weeks. Our preliminary results suggest that MEV treatment of different breastfeeding weeks may induce a faster wound closure but also granulation tissue aberration.

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Development of matrixes and devices on the basis of inert materials by the method of ion-beam treatment to prototype cells and medical targets

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Matrix materials are in demand and promising in the field of cellular technologies. A properly created matrix will not be an inert environment, but an active medium regulating the basic processes of cell life. Created a biomatrix based on inert material for the cultivation of cells from various sources. In the course of the work, factors affecting the increase in adhesion properties were identified, the substrate toxicity and oncogenicity were excluded, the growth of mouse hepatocytes, human fibroblasts and Candida albicans was analyzed on this surface. It is proved: the effectiveness of modifying the surface of the glass to improve the adhesive properties, thus, an increase in biomass in a shorter period of time, the formation of a monolayer of cells. Using scanning electron microscopy, it was found that the 2D matrix has an ordered cellular structure that affects the increased adhesive properties. The 2D matrix is not cytotoxic and oncogenic. Hepatocytes of mice, on a 2D-matrix, are adsorbed in an amount (2.8 ± 0.2) times higher than on a smooth glass. On the treated glass ion formed cell aggregates. The number of human fibroblasts adsorbed on a 2D-matrix is (1.5 ± 0.5) times the number of cells than on a smooth glass. Fibroblasts adsorbed on modified glass have a normal configuration for this type of cell. Candida albicans cells are 5 times more actively adsorbed on the modified glass than on the control glass. Hepatocyte extract of mice incubated on modified glass contains protein fractions with molecular weights of 30, 43, and 120 kDa compared with the control.

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Target optimized variant of CRISPR associated nuclease enables allele-specific knock out of ELANE-related neutropenia

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We present here a novel and broad approach for allele-specific CRISPR gene editing which allows the targeting of different mutated alleles of a disease-associated gene using one CRISPR composition. This approach opens up unlimited opportunities for gene editing therapy in cases of dominant negative mutations, compound heterozygous mutations and haploinsufficiency. First, we analyze human haplotypes and identify either a single nucleotide polymorphism (SNP) or several SNPs that are predicted to be associated with most of the known mutated alleles. Then, we design a CRISPR based gene editing strategy targeting the SNP and optimize a CRISPR associated nuclease (CAS) for that sequence to enable editing only at the desired alleles. We demonstrate the utility of this approach for knocking out mutated alleles of ELANE. Heterozygous mutation in ELANE causes the majority of Severe Congenital Neutropenia (SCN). Over 100 heterozygous mutations in ELANE associate with the disease. We identified three SNPs that can be linked with the majority of mutated alleles and designed CRISPR based strategies to knock out these alleles. Then, we used our directed-evolution platform to optimize a CAS nuclease to effectively and specifically cleave at the SNPs. The optimized specificity of the nuclease allows the allele specific editing. Finally, we show that electroporation of human CD34+ cells with the optimized CRISPR composition knocked out the mutated ELANE allele and the edited cells mature to active neutrophils.

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In vivo genome editing of hAPOC3 in the liver of APOC3 transgenic mouse leads to a robust and stable reduction in serum triglyceride levels and normalization of lipid profiles

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Apolipoprotein C-III (APOC3) is a key player in triglyceride-rich lipoprotein metabolism and is strongly associated with elevated plasma triglyceride (TG) levels. Patients with severe hypertriglyceridemia are at risk for pancreatitis and cardiovascular disease. Loss-of-function mutations in the APOC3 gene are associated with low triglyceride levels and a decreased risk for cardiovascular disease. Meganuclease-mediated genome editing