CD61- or CD61+. This data suggest that the MK progenitors derive from the CD34+CD61- or CD34+CD61+ population. Furthermore, it demonstrates that development into the MK lineage requires progression of CD61 expression, becoming more mature and losing large CFU-MK colony potential and gaining platelet production. We have isolated CB MNCs and cultured the cells on MSCs for 7 days, in media supplemented with SCF, TPO and FLT3L. We detected significant expansion of the CD34+ subpopulations that produced CFU-MK potential and significant platelet production *in vitro*. This MK expansion in co-culture with MSCs may provide significant proliferation of early MKs that could be stimulated *in vivo* to shed platelets and provide faster engraftment times.

220
CLINICAL AND LABORATORY PREDICTORS IMPACTING ALLOGENEIC PERIPHERAL BLOOD STEM CELL MOBILIZATION

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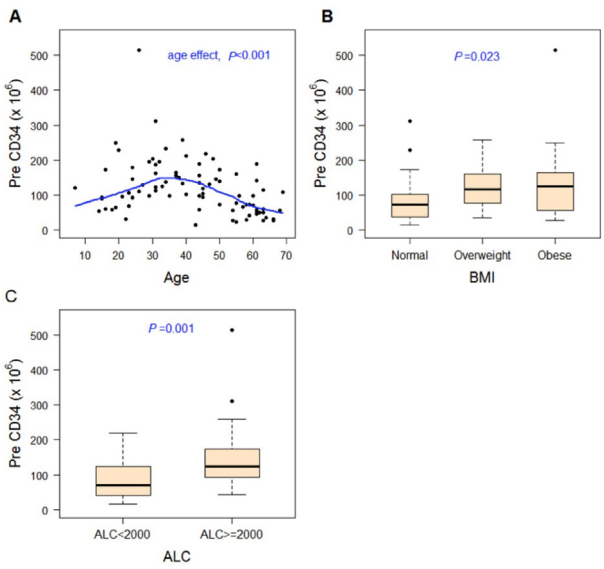
Introduction: CD34+ dose significantly impacts transplant outcomes. Donor characteristics that impact CD34+ mobilization are particularly relevant in haploidentical transplantation, where there may be several eligible donors. A previous study in our institution (Vasu et. al, 2008) reported various predictors of CD34+ yield. We aimed to validate and further understand these predictors.
Methods: We report a single institution retrospective analysis of 86 HLA-identical sibling donors (median age 44 years, range 7-69; 52% female) who underwent G-CSF mobilization between 2006 and 2014. All donors received 10-15 µg/kg/day of G-CSF for 5 days based upon a vial-sparing algorithm, followed by large volume apheresis. Donor circulating CD34 pre-count was used to predict total CD34+ cell yield and determine volume for apheresis.
Results: Univariate analyses evaluated factors such as age, gender, race/ethnicity, height/weight, body mass index (BMI), white blood cell count, platelets, hemoglobin, mean corpuscular volume, absolute lymphocyte counts (ALC), and absolute monocyte counts. After multivariate analysis age, BMI, and ALC were the only factors to retain statistical significance. Age had a nonlinear effect on CD34 pre-count (Figure A). For age< 35, the CD34 pre-count appeared to increase with age; and for age>35, the pre-count decreased with age. Sixty donors between 68-108kg received the same G-CSF dose for mobilization (1080mcg daily X5 days). Of those, donors who were either overweight or obese by BMI yielded a higher CD34 pre-count (p=0.023) (Figure B). Finally, donor baseline ALC greater than 2000 cells/µL was significantly associated with higher CD34 pre-counts compared with ALC less than 2000 cells/µL (p=0.001) (Figure C).
Conclusion: This study validates previously reported findings and highlights the positive correlation between ALC and BMI with the CD34+ pre-count.



221
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222
MESENCHYMAL STEM CELLS PREVENTED THE PROGRESSION OF DIABETIC NEPHROPATHY, IMPROVED RENAL FUNCTION AND MICROVASCULAR ARCHITECTURE IN A CHRONIC MODEL OF TYPE 1 DIABETES MELLITUS

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The most detrimental complication of diabetes mellitus (DM) is diabetic nephropathy (DN) a clinical syndrome comprised of kidney damage and increased risk of cardiovascular disease. Until now, there is no cure for DN; and treatments only help to slow its progression.

The aim of this work was to evaluate the renoprotective effect of mesenchymal stem cells (MSCs) in a chronic model of type 1 DM.

C57BL/6 mice rendered diabetic by the administration of a single high dose of streptozotocin, received intravenously a single dose of MSCs ($0.5x10^6$) at 8 weeks after diabetes induction, since at this time diabetic mice presented functional and structural renal alterations. MSCs renoprotection effect was evaluated 2 and 8 weeks post-administration.

Compared with untreated animals, MSC-treated mice showed a suppressed increase in kidney weight, kidney to body weight index, albuminuria (ELISA), serum creatinine and BUN (enzymatic). These changes were correlated with increased proliferation rate (PCNA), and decreased apoptosis rate (Tunel).

Furthermore, MSC administration restored glomerular capillary surface area (dextran-FITC by confocal microscopy -CM-), and effacement of podocyte foot processes (podoplanin by CM) associated with nephrin expression.

We assessed the level of renal cytokines by qRT-PCR and multiplex array. Compared with normal mice, untreated animals pro-inflammatory molecules were increased and anti-inflammatory molecules were diminished. This pattern was almost normalized in MSC-treated mice. More importantly, the number of infiltrated macrophages (by CM) in kidney was effectively suppressed by MSC treatment.

Although donor cells were found in the kidney of treated mice, their scarcity suggests that the improvement in kidney function was not mediated by the differentiation of MSCs into renal cells, so we suppose the participation of paracrine mechanisms to limit the progression of DN in MSC-treated mice.

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223
INTRAVITREAL ADMINISTRATION OF MULTIPOTENT MESENCHYMAL STROMAL CELLS INCREASES NEUROTROPHIC FACTORS LEVELS, REDUCES OXIDATIVE DAMAGE AND PREVENTS RETINAL NEURONAL CELL LOSS IN DIABETIC MICE

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Diabetic retinopathy (DR) is the most common complication of diabetes and the leading cause of irreversible vision loss. The first symptom is a reduction in color-contrast sensitivity, due to the loss of neuronal cells in the ganglion cell layer of the retina. Available therapies for DR are highly invasive and applicable only in advanced stages of the disease.

Multipotent mesenchymal stromal cells (MSCs) are an attractive tool since they could differentiate into neuronal cells, produce neurotrophic factors and reduce oxidative damage.

Our aim was to evaluate whether the local administration of MSCs lower the loss of retinal neuronal cells in an animal model of DR. For this, mice with type 1 diabetes (T1DM mice) received an intravitreal injection of vehicle or MSCs at a time when pro-damage mechanisms were present but retinal structure still

unaffected. Three months later retinal histology (H&E), gene expression (RT-qPCR) and oxidative damage (ELISA) were assessed.

As expected, T1DM mice presented reduced number of nuclei in the ganglion cell layer of the retina. However, the number of these nuclei in MSC-treated T1DM mice and normal mice were similar. Concurrently, the mRNA levels of *bFGF*, *CTNF* and *NGF* were increased in the eyes of MSC-treated T1DM mice compared with untreated T1DM mice. Additionally retinas from MSC-treated mice had reduced lipid peroxidation and increased antioxidant capacity. Finally, the presence and differentiation of MSCs into neural lineages were assessed 1, 7, 30, 60 and 90 days after injection (flow cytometry and immunohistofluorescence). At any time analyzed, MSCs were detected only in the vitreous cavity and they express no ganglion, astrocyte or pericyte markers.

Thus, the intravitreal administration of MSCs appears as a promising treatment for DR, increasing intraocular production of neurotrophic factors and reducing oxidative damage of the retina, preventing retinal neuronal cell loss.

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224
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225
TROPHIC CORNEAL ULCER TREATED WITH A BIOENGINEERED PARTIAL CORNEA SUBSTITUTE. A CASE REPORT

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Aim: To evaluate a novel bioengineered partial artificial cornea for the treatment of trophic corneal lesions resistant to conventional medical treatment.
Methods: A male patient with a neurotrophic lesion affecting his blind right eye is reported.This patient previously had two cornea transplant procedures. The clinical exploration revealed the presence of endothelial decompensation, optic nerve atrophy due to uncontrolled glaucoma and a partial retinal detachment. In this situation, the patient was selected for a novel experimental treatment based on the surgical implant of a bioengineered human partial cornea in the context of a clinical trial. This cornea model was based on fibrin-agarose biomaterials containing stromal keratocytes within the biomaterial and cornea epithelial cells on top, and it was generated as an advanced therapies medicinal product at the GMP facilities of the University Hospital Virgen de las Nieves, Granada. This cornea model was implanted on the patient's cornea after a superficial keratectomy was performed. Postsurgical evaluation included analysis of visual acuity, graft integrity, wound healing, corneal transparency, neovascularization, inflammation or infection as determined by optical coherence tomography and slit lamp analysis.
Results: The artificial cornea was grafted on the patient's eye surface. A complete healing of the trophic lesion and an improvement of corneal transparency was found. Although visual acuity didnt improve, no signs of inflammation, neovascularization or infection were detected and the patient referred a clinical improvement of his symptoms . As a complication, some bullous lesions were found, which may be explained by the endothelial decompensation.
Conclusion: These results support the clinical use of fibrin-agarose artificial corneas and confirm the biosafety of this type advanced therapies medicinal products.

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226
GROWTH FACTOR BINDING SURFACES FOR IMPROVED CELL CULTURE

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Background: Glycosaminoglycans (GAGs) are be found in the extracellular matrix of many tissues, and play an important role in cell binding, growth and organization processes leading to the establishment and maintenance of tissue structure. In particular, GAGs preserve growth factors in vivo, and enable the release and presentation of these molecules. We have developed a straight-forward surface modification platform for binding GAGs from simple buffer solutions (without chemical modification) which enables their use as a cell culture substrate. The presence of GAGs on a material surface allows the binding and presentation of growth factors, such as FGF-2 and FGF-7 to be enhanced.
Methods: Plasma co-polymerization is a technique capable of producing thin polymeric films with controllable concentrations of chemical functional groups on cell culture surfaces. Using radio-labelling and high-resolution surface analysis techniques, we can show the binding of heparin and other GAGs to the surfaces, and their subsequent capture of growth factors FGF-2 and FGF-7. We culture human primary fibroblasts and keratinocytes on the surfaces with growth factors pre-adsorbed to the matrix, or added separately to the cell culture.
Results and Conclusion: The results show significant improvement in the cell viability from much lower concentrations of growth factor for both FGF-2 and FGF-7 - by as much as two orders of magnitude. This approach has the potential to reduce the amounts of expensive growth factors used for culturing therapeutic cells by the use of novel surface engineering approaches.

227
HEART, BRAIN, EYE AND PANCREAS CELLS THRIVE ON BIOLOGICALLY RELEVANT DEFINED AND XENO-FREE LAMININS

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Laminins are a group of 16 heterotrimeric glycoproteins found in the basement membrane in the extracellular matrix and are composed of α, β and γ chains. Laminins are the only tissue-specific proteins in the basement membrane, are thus the critical factors that differentiate one niche from another and influence the behavior of associated cells, such as adhesion, differentiation, migration, phenotype stability, and resistance to anoikis.

The use of specific laminins for tissue culture and cell therapy applications have been hampered by lack of access to most laminin isoforms. Tissue-purified laminins have been available long but all with poor quality due to protein degradation and impurities. We can now successfully produce human recombinant laminins and show that individual isoforms drastically improve the functional properties of different cells.
Pluripotent Stem Cells: By using LN-521, which is naturally expressed by human PSCs, we can culture stem cells for over 130 single cell passages at split ratios of 1:10-1:30, without any abnormal genetic aberrations and with maintained expression of pluripotency markers.
Cardiomyocytes: By using heart specific laminins, LN-211, LN-221 and LN-521 in the natural combination of adult heart cell expression, the differentiation can be fully controlled in a defined and xeno-free context and with significantly increased numbers of hPSC-derived beating cardiomyocytes.
Neurons and Glia: Neural stem cells prefer LN-521, different neuronal subtypes require primarily LN-511/521 and LN-111 for full maturation, and glia cells express LN-111, LN-211 and LN-521.
RPE Cells: Robust retinal pigmented epithelium cell culture has been reported on LN-521/511, LN-111 and LN-332.

In conclusion, almost all cells grow on specific laminins in the human body and since they are now available as recombinant laminins it makes cell culture in a physiologically relevant environment possible, making production of clinically relevant cells possible.

228
CRYOPRESERVED hMSCs MAINTAIN COMPARABLE IN VITRO FUNCTIONAL ACTIVITY COMPARED TO FRESH hMSCs

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Human bone marrow-derived mesenchymal stem/stromal cells (hMSC) are critical components of tomorrow's cell-based product and devices. Secretion of biomolecules by hMSC influences many biological processes and is thought to be central to the mechanism of action. Since widespread clinical use of hMSC will be facilitated by frozen storage, cryopreserved hMSC must maintain high levels of biological function upon thaw. To address this critical issue we tested the impact of cryopreservation and thawing on the inducible upregulation of IDO by IFN-g and angiogenic cytokine secretion ((VEGF, HGF, TIMP-1 and -2, FGF2, and IL-8) of hMSC: We hypothesized that cryopreserved hMSC would have diminished immunosuppression response and altered cytokine secretion immediately after thaw compared to hMSC fresh from culture. We compared the biological activity of hMSCs (RoosterBio) from 2 donors either (a) straight out of cryopreservation (THAW), or (b) cells that have been in culture for at least 5 days (FRESH) while controlling for PDL. FRESH or