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to the foamed poly(L-lactic-co-ε-caprolactone) 70/30 (PLCL) scaffolds in the TM condition. The PLA 96/4 scaffolds supported the formation of a uniform cell layer in the TM condition compared to the maintenance medium (MM) condition at 2 weeks. The total collagen content and gene expression of tenogenic marker genes of the hASCs was significantly higher with the optimized TM after 14 days. The elastic modulus the braided PLA 96/4 scaffold was more similar to that reported for the native human Achilles tendon tissue. Our study showed that the optimized TM condition supports *in vitro* efficient tendon-like matrix production of the hASCs. The braided PLA 96/4 scaffolds combined with the optimized TM significantly enhanced tenogenic differentiation of the hASCs. The proposed tendon tissue engineering applications represent an emerging approach with potential feasibility.

## 421

### Adipose Tissue-Derived Therapeutic Cells - Towards a Non-Enzymatic Procedure

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Human adipose tissue is an attractive and abundantly available source of adult stem cells applicable in regenerative medicine and tissue engineering. Hence, prerequisite for the translation into clinics is the production of the stromal vascular fraction (SVF) under good manufacturing practice (GMP). A number of companies developed systems aiming for a closed, sterile, safe and reproducible cell isolation process. However, many of these systems are based on enzymatic digestion which is the most expensive part of the isolation process, complicates regulatory authorization and may have negative impacts on cell potency and efficacy. Therefore we compared classical enzymatic cell isolation methods with reduced enzyme concentration methods and a new non-enzymatic isolation method regarding cell yield, identity and potency. Our results demonstrate that enzyme treatment is necessary for applications which require large cell numbers, since reduction of enzyme concentration to 30%, 10% and 0% result in smaller cell yields. In contrast, the viability of the isolated cells as determined via cellular ATP decreased with increasing collagenase concentration. Furthermore, reduction of collagenase concentration showed significant higher adipogenic, osteogenic and chondrogenic differentiation potential. We found that our non-enzymatic method is suitable to isolate therapeutically relevant subpopulations such as endothelial progenitor cells (CD45–/CD31+/CD34+), pericyte-like cells (CD45–/CD31–/CD146+), and supra-adventitial cells (CD45–/CD31–/CD146–/CD34+) with high cellular ATP content and elevated differentiation potency. Cells derived from our new non-enzymatic method showed stronger potential to form tube-like structures. Our findings support the concept of using non-enzymatic closed systems which allow the isolation of therapeutically active cells in a one-step procedure.

## 422

### Exosomes Delivered by Human Cardiac Primitive Cells Impact on Both Cardiac Cellular and Extracellular Compartment

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Although human Cardiac Primitive Cells (CPC) when injected in infarcted heart are not retained by host myocardium, they improve cardiac function. Emerging evidence supports the hypothesis that exosomes may be responsible for beneficial effects induced by stem cells delivered in the infarcted myocardium. Exosomes are nano-sized vesicles naturally secreted by almost all cells and ubiquitously found in cell culture supernatants and biological fluids. Transporting and transferring peptides, lipids, and nucleic acids, exosomes have the potential to modulate signaling pathways, cell growth, migration, and proliferation of recipient cells. Accordingly, CPC may deliver chemoattractive, pro-survival and differentiating signals to resident cells through exosomes. To test our hypothesis, we isolated exosomes released in culture by CPC isolated from adult human myocardium (Exo-CPC) and analyzed the composition of their cargo. Specifically, we searched for the presence of specific factors known to regulate CPC migration, survival and differentiation, as HGF, IGF, TGF, Nkx2.5, Tbx, and Mef2c. Additionally, we tested *in vitro* the potential of Exo-CPC of either regulating CPC proliferation and programmed cell death, and modulating interstitial fibrosis, extracellular-matrix (ECM) synthesis and deposition. Interestingly, on one hand, signals delivered by Exo-CPC affected proliferation and survival of CPC and, on the other hand, regulated ECM proteins production. Therefore, we might speculate that Exo-CPC have potential effect on both resident CPC and fibroblasts when injected in cardiac wall.

## 423

### A Novel Reporter System For Monitoring Of Skeletal Muscle Cell Differentiation

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**Introduction:** Non-invasive monitoring of cell differentiation is an important aspect in cell-based therapeutic strategies that rely on tissue maturity. We have developed a reporter system to track murine skeletal muscle cell differentiation using a tissue specific promoter combined with a secreted bioluminescent reporter.

**Materials and Methods:** To construct a muscle creatine kinase (MCK) specific reporter vector, a secreted bioluminescent (*Metridia* luciferase; MetLuc) or fluorescent reporter (GFP) was placed under the control of the truncated MCK basal promotor enhanced by variable numbers of upstream MCK E-boxes. In addition, seed sequences targeting a set of miRNAs that are known to be downregulated in myogenesis were ligated into the 3′UTR downstream of the reporter gene to reduce signal background. This reporter system allows for convenient and rapid analysis of MCK expression by luciferase assaying of collected supernatant or fluorescence microscopy.

**Results:** The reporter constructs exhibited strong luciferase and fluorescence expression in cells undergoing myogenic differentiation compared to non-muscle cells. In addition, the insertion of seed sequences reduced background signal activity while enhancing signal to noise ratio of the reporters MetLuc and GFP.

**Conclusion:** This reporter system allows kinetic monitoring of cell myogenic differentiation in a non-invasive, highly specific, fast and convenient manner. Moreover, the insertion of seed sequences markedly improves the signal to noise ratio.

## 424

### Bone Marrow-extracted Soluble Factors Maintain Properties of Human Mesenchymal Stem Cells

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Human mesenchymal stem cells (hMSCs) residing in bone marrow stem cell niches are regulated by components of the niche. In this study, we identified soluble factors abundant in the bone marrow niche, characterized their effects on hMSCs, and utilized the soluble factors for hMSC-based tissue regeneration. Our results showed that treatment of bone marrow extract increased hMSC proliferation and reduced cellular senescence. Human MSCs pretreated with bone