of well-defined serum-free and xeno-free media formulations which represent important milestones towards the production of MSC for cellular therapies. In this work, a microcarrier-based suspension culture was explored for the scale-up of MSC expansion in xeno-free medium using synthetic peptide acrylate surface beads. Cells were maintained on Corning® Synthemax® II polystyrene and CELLstart™-coated Solohill plastic microcarriers (dos Santos et al, 2011) for 14 days in xeno-free medium. Bone marrow (BM) derived- and adipose tissue (AT) derived-MSC were seeded at 50,000 cells/mL with 4.5 cm2/mL of microcarriers in spinner flasks (80 mL volume). To maximize cell seeding, the adhesion step was performed in 50% final volume during the first 24 hours. The efficiency of initial cell adhesion to microcarriers was similar for both cell types; slightly better cell adhesion was observed on Synthemax II compared to CELLstart-coated microcarriers: 48% and 43% for AT MSC and 42% to 37% for BM MSC. The homogenous cell distribution on the first days of culture resulted in a higher expansion rate with exponential growth from day 4 to day 6 for AT MSC and day 4 to day 9 for BM MSC. The longer growth phase observed for BM MSC resulted in higher cell densities of 350,000 cells/mL compared to 250,000 cell/mL for AT MSC cultures. Expanded cells maintained their characteristic immunophenotype and multilineage differentiation potential. Ongoing work includes the translation of this culture system to a fully controlled stirred-tank bioreactor (1 L) and the study of the impact of different culture parameters namely feeding regime and dissolved oxygen concentration on cell productivity. This scalable xeno-free, microcarrier-based culture platform is anticipated to enable cost effective and robust production of MSC meeting the needs of the allogeneic "off-the-shelf" MSC therapy sector.

359

DEVELOPMENT OF CELL-ENHANCED CHITOSAN SCAFFOLDS TO OVERCOME LONG GAPS AFTER PERIPHERAL NERVE INJURY

S Wrobel[1,2], M Morano[3], C Meyer[1,2], A Shahar[4], O Ziv[4], K Haastert-Talini[1,2], S Geuna[3], C Grothe[1,2]
[1]Institute of Neuroanatomy, Hannover Medical School, Hannover, Germany, [2]Center for Systems Neurosciences (ZSN) Hannover, Hannover Graduate School for Veterinary Pathobiology, Neuroinfectiology, and Translational Medicine (HGNI), Hannover, Germany, [3]Department of Clinical and Biological Sciences, Neuroscience Institute of the Cavalieri Ottolenghi Foundation (NICO), University of Turin, Turin, Italy, [4]NVR Laboratories LTD., Neural and Vascular Reconstruction, Ness Ziona, Israel

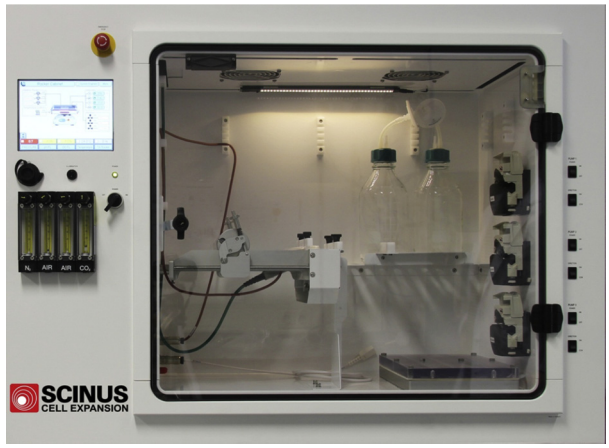
Chitosan-based artificial nerve guidance conduits are developed in the BIOHYBRID project. Composite nerve grafts existing of a chitosan outer shell and a core scaffold made of three-dimensionally structured NVR hydrogel are enriched with genetically engineered rat Schwann cells (SCs). Cell survival, growth factor expression pattern and cellular bioactivity and potency to support functional peripheral nerve regeneration were investigated in vitro and in vivo. In vitro studies demonstrated that selected growth factors (fibroblast growth factor- 2, FGF-2 18kD, and glia-derived neurotrophic factor, GDNF) were over-expressed by genetically engineered SCs seeded into the core scaffold (0.5% NVR gel) in comparison to empty-vector expressing and naïve SCs. Neurite outgrowth of sensory DRG neurons was supported by SCs-FGF-2 and SCs GDNF in co-culture bioassays. The support of neurite outgrowth was comparable to that produced by the same growth factor coupled to ferro-nanoparticles. We further demonstrated that SC viability was preserved when seeded into NVR-Gel/chitosan scaffolds over up to 9 days in vitro (WST-1 assay). During this period, the cells formed aggregates during the first 24hrs but then grew out of these to populate the NVR-Gel scaffold. Encouraged by the in vitro results cell seeded composite nerve guides (naïve SCs, SCs-FGF-2, SCs-GDNF) were transplanted into sciatic nerves of adult rats to bridge 15 mm nerve gaps. Autologous nerve transplantation served as the clinical standard control condition. Over 16 weeks in vivo functional recovery is currently tested by electrophysiology, static sciatic function index motor function evaluation and von-Frey mechanosensitivity evaluation. First in vivo results will be available till April 2014. Our results give a promising perspective of an efficient composite nerve guide for long gap nerve repair. This work has received funding from the European Community's Seventh Frame work Programme (FP7-HEALTH-2011) under grant agreement n° 278612 (BIOHYBRID).

360

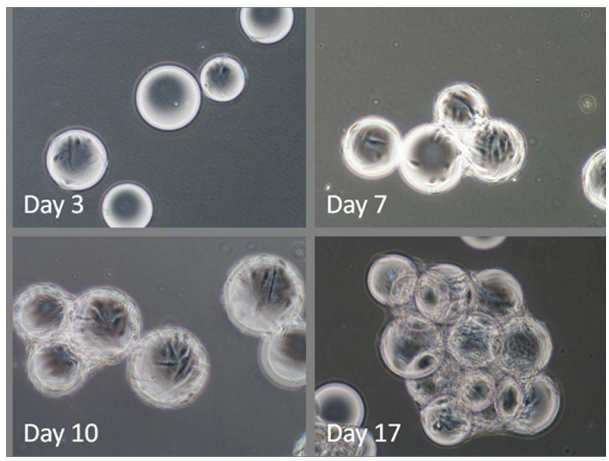
CONTROLLED CULTURE OF ADHERENT CELLS IN A NOVEL, CLOSED BIOREACTOR SYSTEM FOR CELL THERAPY PRODUCTION

R Das[1], R Roosloot[1], W Tra[1], H Roelofs[2], P van Santen[1], J de Bruijn[1,3]
[1]Xpand Biotechnology BV, Bilthoven, Netherlands, [2]Hematology and Blood Bank, Leiden University Medical Centre, Leiden, Netherlands, [3]Twente University, Enschede, Netherlands

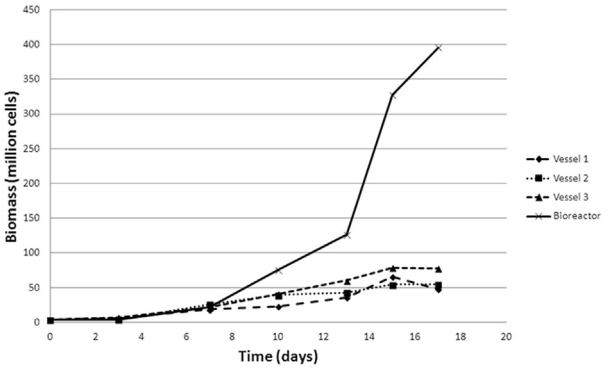
There is an increasing need to make adherent cell expansion cheaper, safer and easier. Standard culture practice should be translated to closed, controlled systems to increase safety and quality. Current closed systems are limited in their culture range, only limited maximal cell numbers can be obtained, or the minimal volume is too large to support growth of small cell numbers. Therefore, a bioreactor was designed that supports growth of limited cell numbers to therapeutic quantities. BM-MSCs (P2) were expanded under controlled conditions (DO 25%, pH 7.3) in a closed bioreactor system (Fig 1). MSCs were grown for 17 days on microcarriers in an expandable culture bag and compared to stirred vessel culture. Flow cytometry was performed and differentiation capability was assessed. Therapeutic potential will be assessed through stimulation assays using various cytokines (e.g. IDO production upon IFN-γ stimulation). Using closed procedures, culture was maintained for 17 days (Fig 2) and volume was expanded twice, resulting in a final volume of 850 ml and 5.0E5 cells/ml (400E6 cells, Fig 3), whereas stirred vessels yielded 60*106 cells (PDL 6.8 vs 4.1). Cells were positive (>95%) for CD73, CD90 and CD105 and negative (<2%) for CD43, CD11b, CD19, CD45 and HLA-DR. All cells could be differentiated along the osteo- and adipogenic lineage. However, immunesuppressive potential needs to be assessed. A novel



Xpand's bioreactor system.



MSCs were successfully cultured inside a bioreactor bag for 17 days. Therapeutic cell numbers were obtained at harvest.



Cell count of bioreactor culture versus three stirred vessels. Cells in the bioreactor doubled 6.8 times, while the stirred vessel controls resulted in only 4 population doublings. A total of 400 million cells were obtained.

bioreactor system with sampling possibilities is introduced for GMP-grade MSC production. The system enables culture from minimal numbers to therapeutic quantities in a controlled environment. The closed design reduces contamination risk and enables operation outside expensive cleanroom facilities. Operator involvement is minimized, limiting labor and production costs. The switch from open, uncontrolled procedures to closed systems for production of cell therapy products presents a major step forward to improved quality, reproducibility and affordability of these therapies.

361
HUMAN-DERIVED RAW MATERIALS: CONTROLLED, CONSISTENT COLLECTION AND CRYOPRESERVATION ENABLE SUCCESSFUL MANUFACTURING OF CELL-BASED PRODUCTS

TV Ramos[1], W Wang[1], J Okhovat[1], G McPherson[1], S Burger[2]
[1]HemaCare Corporation, Van Nuys, California, United States, [2]Advanced Cell & Gene Therapy, LLC, Chapel Hill, North Carolina, United States

Human cells are critical raw materials for manufacturing cell therapy products, but often introduce significant variability. Rigorous operational controls and quality systems, however, enable optimal collection of high-quality, consistent cellular material. HemaCare, a long-standing supplier of human-derived blood components, controls apheresis procedures and collection sites under a formal quality system, with GMP-compliant, validated procedures and equipment, and GTP-compliant donor screening and tracking. HemaCare performed 86,799 cellular apheresis collections in the last seven years (year ending July 31, 2013), including patient and normal-donor peripheral blood mononuclear cells (MNCs), and plateletpheresis products for research, clinical trials, and commercial products. HemaCare's unmobilized apheresis products showed consistently high MNC purity, with 93.8% of products containing $\geq$75% MNC, and an average of 85.66% MNC $\pm$ 7.1% (mean $\pm$ 1 SD). Red blood cell contamination was low, with hematocrit averaging 1.78% $\pm$ 0.7%. Approximately 85% of HemaCare donors have donated apheresis products 5 or more times; this repeat-donor pool also contributes to product consistency. HemaCare's laboratory is equipped with Miltenyi Biotec technology for isolation of cellular raw material into purified cellular subpopulations. The consistency and viability of the purified products are measured with flow cytometry. Using BioLife Solutions' serum-free and protein-free fully-defined cGMP CryoStor™ cryopreservation medium with purified cells, post-thaw recovery rates of cell fractions have been above 95%, based on 7AAD staining. Dendritic cells and macrophages have demonstrated post-thaw recovery rates of $\geq$90%. CryoStor™ cryopreservation medium, in combination with freezing in the BioCision CoolCell™ freezing container, has enabled HemaCare to standardize the cryopreservation process, reducing variability while optimizing post-thaw viable cell recovery of its research products.

362
DEVELOPMENT OF A SCALABLE MANUFACTURING PROCESS FOR BONE-MARROW DERIVED HMSC'S IN A LOW-SHEAR SINGLE-USE BIOREACTOR SYSTEM

D Giroux[1], **P van der Aar**[2], M Serra[3]
[1]PBS Biotech Inc., Camarillo, California, United States, [2]DYNC, Breda, Netherlands, [3]IBET, Lisbon, Portugal

Daniel Giroux, PBS Biotech, Inc. USA, dgiroux@pbsbiotech.com Margarida Serra, Instituto de Biologia Experimental e Tecnologica (iBET) Marcos Sousa, Instituto de Biologia Experimental e Tecnologica (iBET) Bone-Marrow derived Mesenchymal Stem Cells (BM-MSCs) are multipotent non-hematopoietic cells. They are easy to isolate, grow well in vitro, and possess both immunomodulatory and low immune reactivity properties. MSCs are an ideal cell for the development of "off the shelf" allogeneic cellular therapeutics, To move from these small trials to commercialization, cells must be manufactured at large scale while remaining potent and safe. To address this manufacturing bottleneck, we have developed a unique MSC manufacturing process where BM-MSCs are cultured on microcarriers with low shear mixing using the Air-Wheel Bioreactor. BM-MSCs (StemCell Technologies) were cultured in xeno-free Mesencult medium (StemCell Technologies) on Synthemax II microcarriers (Corning) in a PBS 3 Air-Wheel Bioreactor. Processes for microcarrier seeding, expansion via bead-to-bead transfer, and harvest of MSCs all took place within the Bioreactor. Cell growth and metabolic rates were monitored, and a traditional stirred-tank bioreactor was run in parallel as control. In the Air-Wheel Bioreactor, the cells attached to the microcarriers faster and reached confluence more quickly than a stirred-tank bioreactor. MSCs cultured as described met the International Society of Cellular Therapy (ISCT) minimum criteria for BM-MSC (> 95% CD73, CD105, CD90 and < 2% CD34, CD45, CD14, CD19, HLA-DR) and tri-lineage differentiation. In this study we show that BM-MSCs can be expanded on microcarriers using a low-shear Air-Wheel Bioreactor with bead-to-bead transfer that is amenable to scale up to commercial manufacturing. When BM-MSCs are cultured using this novel expansion system, microcarrier loading is fast and uniform, the MSCs are highly proliferative, and they retain the characteristic markers of BM-MSC.

363
DEVELOPMENT OF A NEW ADVANCED THERAPHY MEDICINAL PRODUCT FOR BONE REGENERATION TREATMENT; FROM BENCH TO BEDSIDE

M Caminal, N Pujals-Fonts, J Vives, M Codinach, I Oliver-Vila, A Casamayor-Genesca, M Blanco, R Coll, A Pla, J Garcia
XCELIA, Banc de Sang i Teixits, Barcelona, Barcelona, Spain

Purpose: In the bone regeneration context, tissue engineered products provide a significant advance in new emerging therapeutic options for the treatment of bone injuries as an alternative to the classical techniques (autologous bone graft, distraction osteogenesis or allogenic bone). Here we present the development of a new advanced therapy medicinal product (ATMP) composed of *ex vivo* expanded mesenchymal stromal cells (MSC), loaded onto bone scaffolds and shaped as the lesion to treat using fibrin sealant, from preclinical studies to phase I/IIa clinical trials.

Materials and methods: Three preclinical studies were performed: - Two large animal studies were conducted in order to evaluate safety and efficacy of the ATMP. The sheep models chosen were 1) osteonecrosis of the femoral head and 2) Critical size segmental bone defect. - A small animal model was carried out to evaluate tumorigenicity after subdermal implantation of the ATMP. Large number of MSC (15-30x10^6) were expanded from bone marrow mononuclear cells and immobilized in a bonny scaffold. This process was developed and patented by XCELIA (Procedure for obtaining a tissue engineering product for the regeneration of bone tissue, EP 2361971 A1). Currently, two clinical trials are undergoing: 1) Mesenchymal Stem Cells in Osteonecrosis of the Femoral Head (NCT01605383) and 2) Safety Study of Mesenchymal Stem Cells and Spinal Fusion (NCT01552707). Some patients have already been treated and the recruitment process is still open.

Results and conclusions: Preclinical studies in both large and small animal models confirmed the safety of the ATMP and evidenced its efficacy. A total number of eleven batches were GMP manufactured to date, and no adverse effects have been found in treated patients. Developed ATMP has promising clinical results treating both osteonecrosis and spinal fusion bone regeneration.

364
ADVANCED CELL THERAPY INDICATED FOR GONARTHROSIS TREATMENT: THE WAY TO THE COMPLETED CLINICAL TRIAL