skeletal tissue engineering. Conventionally, well-developed osteogenic cells have been considered ideal for implantation, but the use of immature progenitor cells has recently shown good success. We have used multi-layered human periosteal sheets (PSs) in more than 80 cases in our periodontal regenerative therapy and observed appreciable regeneration of alveolar bone. Very recently, we have used a stem-cell medium to develop more multilayered PSs *in vitro*. In this study, we characterized these PSs histologically, biochemically and biomechanically.

Materials and Methods: Periosteum tissue was excised from young volunteers' alveolar bone, cut into small segments, and explant-cultured without enzymatic dispersion using a stem-cell medium, MesenPRO (Invitrogen). Expression of surface antigens, mRNAs and proteins were examined. Elastic modulus was evaluated by nanoindentation using an atomic force microscopy. In animal implantation studies, PSs implanted subcutaneously into nude mice were examined histochemically and radiologically, and PSs labeled with a NIR dye were tracked by *in vivo* imaging.

Results: PSs expanded with MesenPRO contained a large population of immature $CD146^+$, ALP^- progenitor cells that extruded a thick ECM containing the collagens and proteoglycans. Minor integrins and cell adhesion molecules were upregulated, but major integrins involved in osteogenic differentiation, such as integrins $\beta1$, $\alpha1$ and $\alpha3$, were downregulated. Cells on the sheet surface were more flexible than dispersed cells cultured on polystyrene dishes. Although being less maturation *in vitro*, these PSs survived longer and showed higher osteogenic activity with upregulated ALP once implanted into nude mice.

Discussion: These thicker PSs provide a flexible environment for their cells and thereby possibly maintain them at immature stages *in vitro*. Because of increased expansion of immature progenitor cells, these PSs function as a more osteogenic grafting material at the implantation site.

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GAIN YIELD WITH REDUCED MANPOWER BY USING CLOSED ATMI'S XP BIOREACTOR TECHNOLOGY

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Promethera Biosciences® is a Belgian Biotech company, producing the cell therapy product, HepaStem, to treat serious metabolic liver disorders. This treatment is based on human heterologous liver progenitor cells isolated from healthy adult livers. An European phase I/II clinical trial is currently ongoing for the treatment of Crigler-Najjar syndrome and Urea Cycle Disorders in a pediatric setting. To further upscale the process, minimize manual operations & related-risks, as well as to reduce overall costs, Promethera has developed a fully closed system.

Currently, the manufacturing process is performed in open aseptic conditions using CellBind-treated CellStacks. The next generation production process will be based on ATMI's Xpansion 2D multiplate bioreactor technology. This multiplate bioreactor offers a similar surface treatment & area, allowing for an easy transfer from the existing multi-tray stacks process. But in addition, it offers a fully controlled environment via real-time monitoring of temperature, dissolved oxygen, pH and media flow rate, reducing in-process variations. Monitoring of cell morphology is accomplished via Ovizio's digital holographic microscopy.

To date, Promethera's liver progenitors were successively expanded in XP10 (6120 cm^2), XP50 (30600 cm^2), and XP180 (110160 cm^2) without change in growth rate, cumulative population doubling time, homogeneous distribution, and cell quality (identity/purity and potency). A substantial increase in cell yield was achieved.

In conclusion, the Xpansion™ 2D multiplate bioreactor offers a valuable technology for large-scale production. This technology results in reduced costs and increased batch-to-batch consistency. It allows reduction of manual handling & manpower and improvement of in-process control. Using this single-use technology, Promethera is able to increase the yield of its production process by >20-fold and reduce manpower by 50%.

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ADVANCING APPROACHES FOR BONE REGENERATION USING FRESHLY SHIPPED MARROW HUMAN MESENCHYMAL STROMAL/STEM CELL PRODUCED INTO SEVERAL EUROPEAN CGMP FACILITIES

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Investigations on Mesenchymal Stromal/Stem Cells (MSC) have characterized their tissue regenerative potency, encouraging their introduction in regenerative medicine applications, trough the development of several animal models. These results also highlighted several key limitations, among them we focused on appropriate shipment conditions. Since cGMP facilities, where MSC expansion takes place, may be far from the operating theatres, it is important to understand the impact of cell transportation on functional outcome in vivo. To explore the influence of transportation on MSC function, we develop a xenograft in NOD SCID model by human bone marrow (BM)-MSC. We mimicked shipment for 18 hours at 4°C, used cell densities equivalent for therapeutic use to test the influence of three clinical grade transportation buffers (0.9% saline +/- 4% albumin from two independent sources) versus cell maintenance medium. Cell viability post shipment conditions was >80% in all cases allowing evaluation of the following cGMP-MSC phenotypes: proliferation rate, adhesion to plastic and hydroxyapatite tri-calcium phosphate osteoconductive biomaterial (HA/ß-TCP 3D), ex vivo osteogenic differentiation in contexts of 2D monolayers and HA/ß-TCP 3D scaffolds, and in vivo ectopic bone formation after subcutaneous implantation of cells with HA/ß-TCP scaffold into NOD/SCID mice. A transportation temperature close to 4°C was key for maintaining good cell viability and function. Among the compared transport buffers, no significant differences were observed for the parameters evaluated and when cells were kept cold, addition of human albumin didn't necessarily improve MSC phenotype but reduced MSC performance. In conclusion, addressing the challenges facing regenerative medicine today, we have shown that BM-MSC can sustain clinical grade shipping conditions making bone in vivo. These pre-clinical results provide optimism for continued improvements in translational regenerative medicine and expand the potential clinical use of MSC.

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A CRYOPRESERVATION SYSTEM FOR DIRECT CLINICAL USE OF MSC

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For allogeneic "off-the-shelf" clinical transplantation or transfusion applications, a large number of frozen-stored MSC are usually required. Cryopreservation of these cells at concentrations commonly used in therapeutic procedures generates large product volumes containing undesirable quantities of DMSO. Several strategies have been tested to reduce the DMSO volume in the graft, such as washing and concentrating cells after thawing, freezing at higher cell concentration, using reduced DMSO concentration, or fractionated infusion of cells over longer time. Cell washing after thawing usually requires methods involving undesired mechanical forces and osmotic stresses causing cell clumping and potential significant cell loss. The aim of the current study was to determine if cryopreserving a higher concentration of MSC at reduced DMSO concentration could have detrimental effects on the viability, stem cell characteristics and in vivo engraftment potential of adipose derived stem cells (ASC).

As a cryopreservation container, CellSeal® closed system cryogenic vial was used. The DMSO concentration for freezing was adjusted to a final concentration of 5% v/v. Cells were cryopreserved using a controlled rate freezer to -80°C and transferred to LN2 for long term storage. The thawed cells were diluted 50% to a final DMSO concentration of 2.5% and analyzed for viability