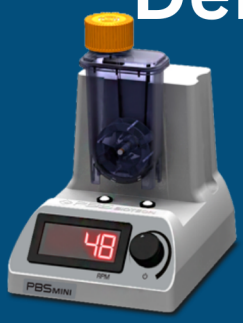
WHITE PAPER | PBS-0.1 MINI S.U.B.

Defined Biomatrices Accelerate MSC Manufacturing Across Scales



INTRODUCTION

Cell-based therapies are promising medical treatments for a broad range of diseases. Predominantly, stem cells are utilized for those treatments, which are sourced either patient-specific for autologous therapies or from healthy donors as part of allogeneic therapies. The advantage of an allogeneic approach is the possibility to expand cells to clinically relevant numbers independent of the patient's medical state, therapy time point, and location. Moreover, the establishment of stem cell banks comprising different donors allows for timely therapy and reduced manufacturing costs. Irrespective of the stem cell source, adverse immune reactions must be prevented for any cell-based therapy for patient safety and treatment outcome. A variety of clinical studies show the safety of mesenchymal stem/stromal cells (MSCs) for the treatment of various diseases due to their low immunogenicity (Wang et al, 2021). MSCs inherently bear regenerative properties and modulate the immune system, mainly through numerous soluble immunomodulatory and trophic factors, different types of subcellular vesicles, and efferocytosis mechanisms (Weiss and Dahlke, 2019; Jovic et al., 2022). The distinct modes of action allow for the therapeutic application of MSCs, e.g., osteoarthritis, ischemic stroke, multiple sclerosis, liver failure, or Crohn's disease. Although promising results have been shown for many of these clinical applications (Yu et al., 2021; Cui et al., 2022; Freitag et al., 2022; Yao et al., 2022), improving the therapeutic efficiency of MSCs is still challenging.

The number of MSCs necessary for an effective treatment is determined for each indication individually. In clinical trials, effective doses range from 70 to 190 million cells per patient (Kabat et al., 2020). Thus, a safe and efficient manufacturing process is required to meet the demand of high-quality MSCs for therapeutic applications. A manufacturing process starts with the isolation of MSCs from the tissue of origin, continues with their expansion in a 2D monolayer culture and is potentiated by a final expansion in a bioreactor.

Hence, manufacturing optimization comprising each of the aforementioned processing steps is required to produce high-quality MSCs in a cost-effective manner. denovoMATRIX has developed cell- and process-specific biomatrices that recreate important aspects of the extracellular matrix of MSCs to improve their isolation efficiency from the tissue source and to enable their long-term serum-/xeno-free expansion in a 2D monolayer (Thamm et al., 2020, 2022). Since monolayer expansion is labor-, cost- and space-intensive, denovoMATRIX transferred their biomatrix technology onto microcarriers that can be used in 3D bioreactor setups. In this study, we recreated the manufacturing process of an MSC-based product in small scale starting from the isolation of MSCs from cryopreserved human bone marrow mononucleated cells (BM-MNCs) using the isoMATRIX. We examined their monolayer expansion on the myMATRIX MSC and their culture on beadMATRIX in a suspension bioreactor setup. For the latter, we used the PBS-0.1 Mini Vertical-Wheel® Bioreactor (PBS-0.1 Mini) that is optimized for culturing shear stress sensitive cells on microcarriers thereby eliminating the need for anti-foaming agents or shear protectants.

RESULTS

isoMATRIX increases isolation efficiency

The isolation of MSCs from their tissue of origin is extremely crucial for the efficiency of the entire manufacturing process. By choosing an optimal microenvironment for the isolation process the clonogenic and proliferative potency can be drastically increased (Thamm et al., 2022). Here, we isolated MSCs from cryopreserved BM-MNCs from two donors using the isoMATRIX in combination with RoosterNourish-XF medium (RoosterBio) and compared it to a competitor cultureware designed for improved cell attachment. We isolated approximately 50% more MSCs from donor 1

and 170% more from donor 2 compared to the competitor product (Fig. 1A). The isolated MSCs showed high viability (Fig. 1B) and the typical fibroblast-like morphology (Fig. 1C). The isolation efficiency (number of MSCs obtained per MNC) was 0.2 MSCs/MNC for the isoMATRIX on average and 0.1 for the competitor product. Interestingly, only Thamm et al. 2022 have so far reported an isolation efficiency >0.1 in a xeno/-serum-free medium. These results indicate that the ECM-mimicking formulation of the isoMATRIX improves isolation efficiency of BM-MSCs.

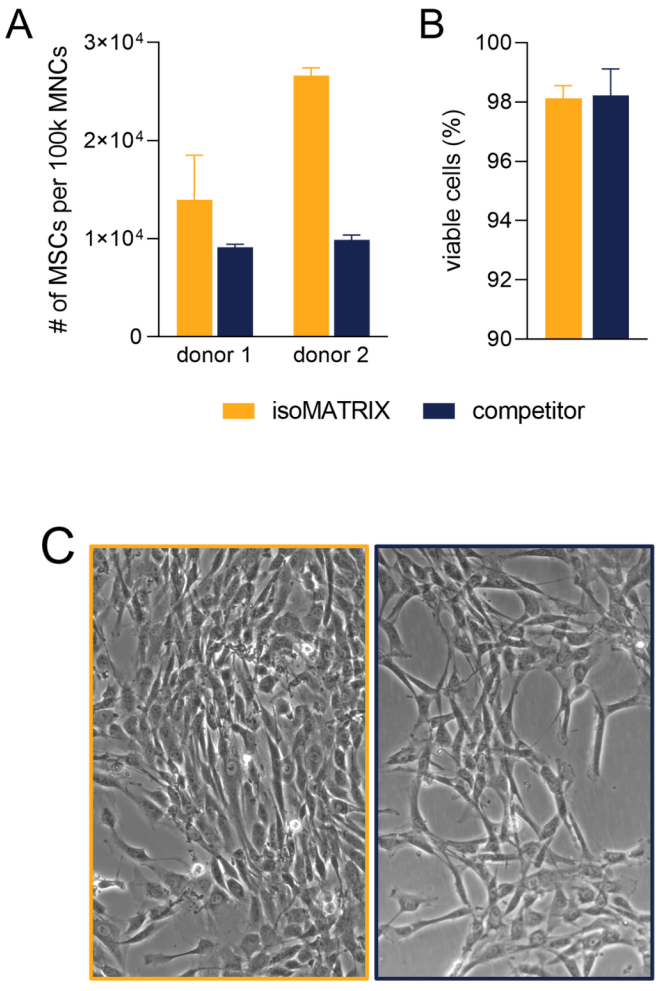


Figure 1. Isolation of BM-MSCs from cryopreserved BM-MNCs. Cryopreserved BM-MNCs were gently thawed and seeded for the isolation of MSCs either on isoMATRIX or a competitor product in combination with RoosterNourish-XF medium. Inoculation density for donor 1 was 120,000 MNCs/cm² and for donor 2 60,000 MNCs/cm². (A) Number of isolated MSCs per 100k MNCs (mean of cell count±SD) and (B) viability of MSCs was determined at day 10 (donor 2) or day 11 (donor 1) post inoculation (mean of donors±SD). The photographs (C) were taken at 10X on day 10-11 to represent cell morphology of donor 1 on isoMATRIX (left panel) and donor 2 on competitor product (right panel).

Efficient 2D cell expansion on myMATRIX

Once MSCs have been successfully isolated from their tissue of origin, MSC manufacturing for clinical applications usually continues with 2D in vitro expansion of the isolated cells. Several major obstacles are encountered in the expansion phase: 1) expansion capacities, 2) maintenance of MSC identity, and 3) preservation of proliferative capacities. Here, cells obtained from the isolation with isoMATRIX were expanded for 5 passages either on myMATRIX MSC or the competitor product designed for improved cell attachment. We performed a detailed analysis of passage 2 starting with the determination of cell density of the two donors after 24 hours (Fig. 2A). We observed a 33% higher cell density when culturing the cells on myMATRIX MSC (~ 3,100 cells/cm²) compared to the competitor product (~ 2,100 cells/cm²). Interestingly, the cell density on myMATRIX MSC exceeded the seeding density of 2,500 cells/cm² indicating that the MSCs have recovered from the seeding procedure and already started to proliferate. We also generated growth curves over the 5-day culture period for both donors (Figs. 2B and C). Donor 1 showed enhanced proliferation on myMATRIX MSC compared to the competitor product while donor 2 grew comparably good on both surfaces. Thus, the analysis of passage 2 indicates an enhanced cell recovery from the seeding procedure on myMATRIX MSC and an improved support of one of the tested donors. During the entire expansion over 5 passages, the performance of myMATRIX MSC was comparable to the competitor product for both donors (Fig. 2D and E) and hence, ensuring an efficient cell expansion.

beadMATRIX supports rapid MSC proliferation in bioreactor setup

To manufacture relevant MSC doses of around 10^8 cells/patient, cell expansion in a bioreactor setup is the safest and most cost-effective way due to strongly reduced manual handling. However, in addition to the challenges in 2D expansion, bioreactor culture poses shear stress that can affect MSC attachment and growth negatively. In this study, we used the PBS-0.1 Mini that provides a homogeneous culture environment while using a gentle and efficient agitation mechanism to mitigate the risks of shear stress. MSCs obtained from the primary culture of MNCs on isoMATRIX were further expanded for 2 passages as 2D seed cultures either on myMATRIX MSC or the competitor product designed for improved cell attachment. After passage 2, both cell populations were seeded either on beadMATRIX or a competitor product of microcarriers in PBS-0.1 Mini using an inoculation density of 25,000 cells/ml. Irrespective of the culture surface during the 2D seed train expansion phase, MSCs showed comparable proliferation on both microcarrier types (Fig. 3A and B).

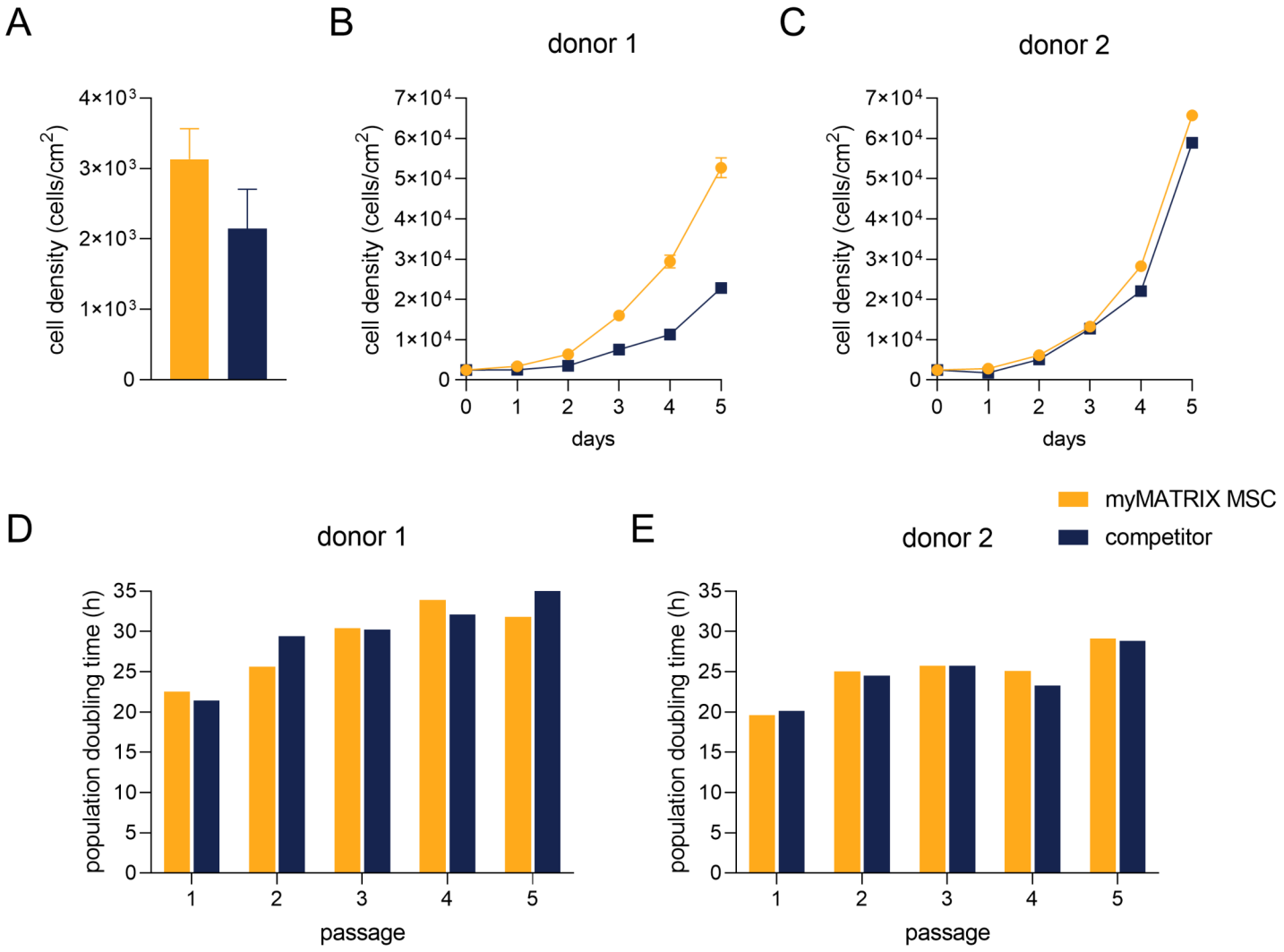


Figure 2. BM-MSC attachment and proliferation in monolayer culture. After primary culture of BM-MNCs to derive MSCs, cells were expanded for 5 passages either on myMATRIX MSC or a competitor product with RoosterNourish-XF medium. (A) 24 h post-seeding, cell density was determined for both donors (mean of both donors±SD). Growth curves of passage 2 of donor 1 (B) and donor 2 (C) cells are depicted. For each passage, the population doubling times were calculated and are illustrated in (D) for donor 1 and (E) for donor 2 cells.

CONCLUSION

In this study we performed a manufacturing process from cell isolation to monolayer expansion to the final bioreactor setup in a small scale. For the first time, we show that using a combination of isoMATRIX, myMATRIX MSC and beadMATRIX improves the manufacturing process with highly enhanced isolation efficiency and robust 2D and 3D cell proliferation. The isolation efficiency of isoMATRIX was on average doubled compared to the competitor 2D product while myMATRIX MSC and beadMATRIX showed comparable, if not greater, cell expansion compared to their respective competitor product. Thus, the manufacturing time of a specific number of cells can be massively reduced or more cells could be manufactured in the same time frame by the use of denovoMATRIX products (Fig. 4). Moreover, it has been demonstrated that the PBS Vertical-Wheel® (VW) bioreactor family supports scale-up of MSC production process from 0.1 L up to 50 L working volume in a highly consistent manner (unpublished data). In sum, therefore, the MSC-specific biomatrix system developed by denovoMATRIX in combination with the PBS VW Bioreactors at various scales (0.1 L – 80 L) will enable cost-effective and reliable manufacturing processes of MSCs for therapeutical applications.

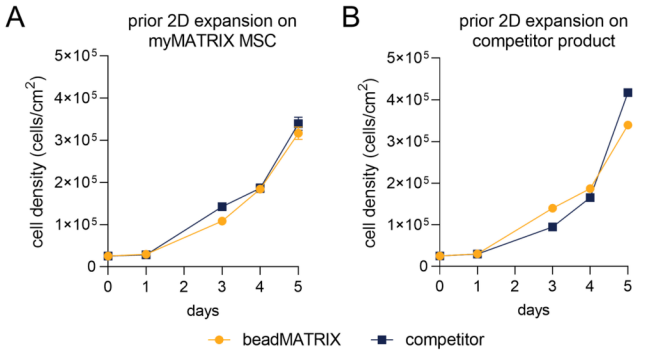


Figure 3. 3D expansion of BM-MSCs in microcarrier-mediated bioreactors. After isolation and expansion for 2 passages, donor 2 cells were cultured on beadMATRIX vs. a commercial product of microcarriers (developed for MSC culture) in combination with RoosterNourish-XF medium in PBS-0.1 Mini. Moreover, in order to test the influence of the 2D culture surface from the 2D seed train expansion phase on the cell growth during the subsequent microcarrier culture, MSCs expanded on myMATRIX MSC (A) or a competitor product (B) were expanded on both beadMATRIX and the microcarriers from a competitor.

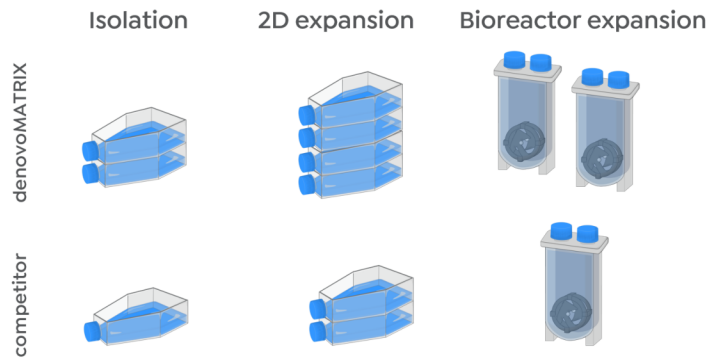


Figure 4. Illustration of the cell yields of an MSC-based therapeutic product based on our study. The manufacturing processes comprise the isolation of MSCs (consisting of isolation and initial expansion), a monolayer expansion step (also known as a 2D seed train culture) and the final cell proliferation in PBS-0.1 Mini. Using denovoMATRIX products that are specifically designed to recreate important aspects of the microenvironment of MSCs doubles the cell isolation efficiency during the primary culture of bone marrow cells. In combination with a robust expansion in the monolayer culture and bioreactor setup, the denovoMATRIX system facilitates the manufacturing of relevant cell numbers in a shorter time frame or at least twice the number of cells in the same time.

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