Reliable and Robust Animal-Component-Free hMSC-BM Expansion on Ready-to-Use CCCadvanced® FN1 motifs Surface

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Abstract

In the last decade, human mesenchymal stem cells (hMSCs) have generated increasing scientific interest. Prior to their use as a powerful tool for research applications, hMSCs must be expanded without losing their phenotypic properties. This requires stable and completely defined hMSC culture systems consisting of a combination of growth surface and culture medium. Made up of RGD-derived motifs supporting cell attachment, the CCCadvanced FN1 motifs surface from Eppendorf represents a completely synthetic surface for hMSC cultivation in animal-component-free culture conditions. This surface combines convenience with reliable hMSC cultivation: the ready-to-use consumable significantly reduces labor time and effort for scientists while offering a fully synthetic growth surface with a high level of consistency during long-term hMSC expansion.

Introduction

hMSCs consist in a heterogeneous population of multipotent cells isolated from various tissues [1].

Their specific properties such as mesodermal differentiation potential, immunomodulation and secretion of anti-inflammatory molecules make them a promising stem cell population in various basic and applied research applications [2].

Present at relatively low abundance in their tissue of origin, hMSCs require a robust *in vitro* expansion process to obtain sufficient high-quality cell numbers. Traditionally, hMSCs are expanded *in vitro* in presence of serum on a tissue culture-treated (TCT) surface.

Nevertheless, the common use of animal-derived materials such as serum presents several drawbacks [3]. In the absence of serum proteins, hMSCs require additional cell adhesion-promoting coating on the culture surface, but frequently used coatings of biological origin are inherently complex and non-defined, which might impact experimental reproducibility. The FN1 motifs surface is made up of synthetic RGD-derived motifs, specifically designed to mimic the cell attachment site of native extracellular matrix proteins such as fibronectin. Used in combination with synthetic culture medium and dissociation solution, this surface represents an effective synthetic alternative to biological coatings. Being ready-to-use, it constitutes a real improvement for researchers, reducing labor time and effort while offering a better lot-to-lot consistency and reliable performances in comparison to self-coating solutions. Here we show that the FN1 motifs surface is highly suitable for successful short-term and long-term hMSC-BM expansion in combination with various xeno-free media.*

Results and discussion

Efficient short-term hMSC-BM expansion in various xeno-free culture media

The FN1 motifs surface supports efficient hMSC-BM growth in combination with different xeno-free (XF) culture media (Fig. 1). In a traditional serum-containing culture system, hMSCs adhere and proliferate similarly on the FN1 motifs surface and on TCT. On both surfaces, cells exhibit their typical fibroblast-like morphology. In absence of serum, hMSCs present difficulties to adhere and proliferate on the TCT surface, suggesting the requirement of additional cell adhesion-promoting coating.

By contrast, the FN1 motifs surface efficiently supports hMSC attachment and growth, whatever the media tested. Cells show elongated spindle-shaped cell morphology classically observed when expanded in XF culture conditions [4].

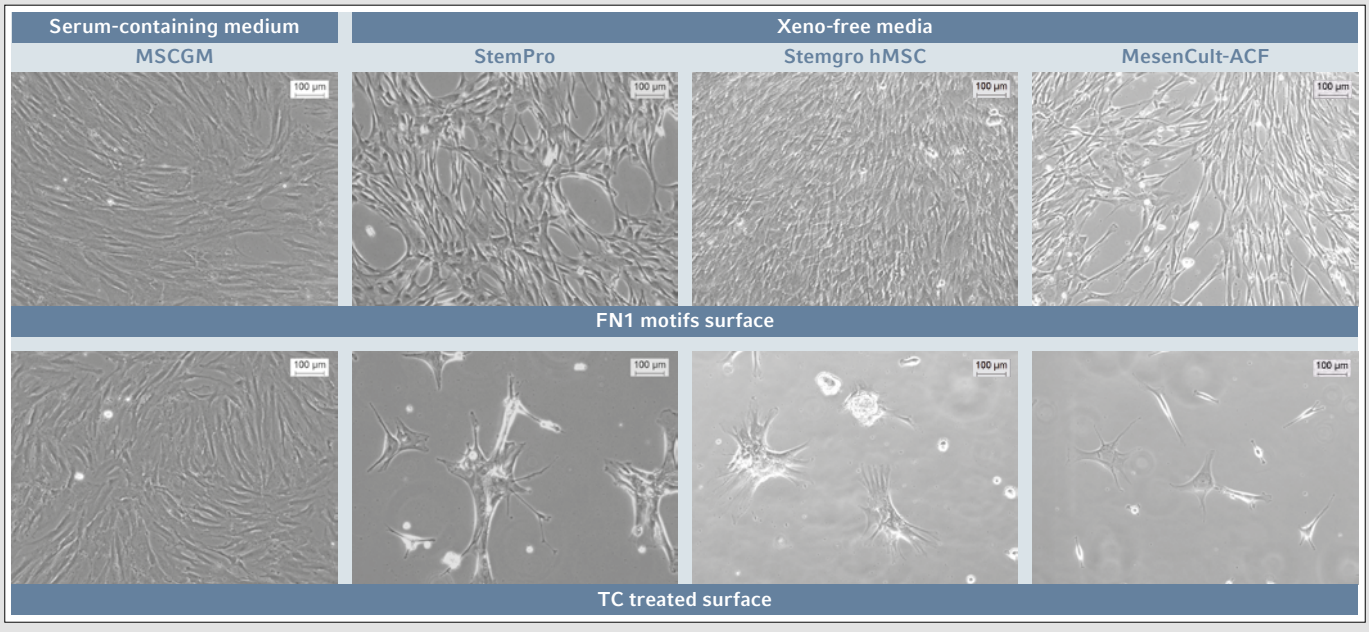

Fig. 1: hMSC-BM morphology after short-term expansion on CCCadvanced FN1 motifs surface in different culture media

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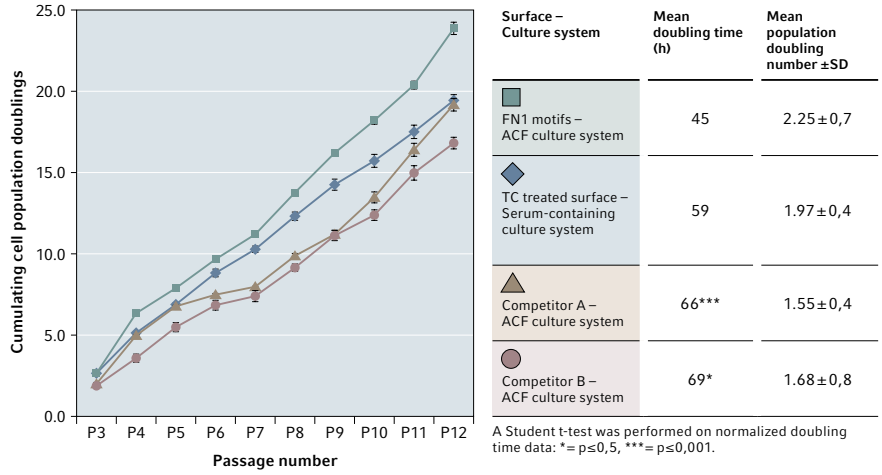

Surface – Culture system	Mean doubling time (h)	Mean population doubling number ±SD
FN1 motifs – ACF culture system	45	2.25±0,7
TC treated surface – Serum-containing culture system	59	1.97±0,4
Competitor A – ACF culture system	66***	1.55±0,4
Competitor B – ACF culture system	69*	1.68±0,8

A Student t-test was performed on normalized doubling time data: *= p≤0,5, ***= p≤0,001.

Fig. 2: hMSC proliferation rate during long-term expansion in different animal-component-free culture systems

Robust long-term hMSC-BM expansion in a completely defined, synthetic culture system

To confirm that the FN1 motifs surface supports long-term hMSC-BM expansion in a completely synthetic culture system without impacting cell quality, cells were maintained during 10 successive passages on this surface. In parallel, cells were cultured on two other synthetic surfaces (ready-to-use Competitor A and self-coated Competitor B). As a reference, cells were expanded in a traditional culture system (TCT, serum-containing medium and Trypsin/EDTA). The proliferation rates show that the FN1 motifs surface supports robust and stable hMSC proliferation through the entire culture period (Fig. 2). As compared to the other experimental conditions, cells expanded on the FN1 motifs surface present a significantly faster proliferation rate with short doubling time and high population doubling number.

To confirm the characteristic hMSC multipotency maintenance after long-term expansion on the FN1 motifs surface in a synthetic culture system, the ability of expanded cells to differentiate *in vitro* into osteogenic, adipogenic and chondrogenic lineages was assessed.

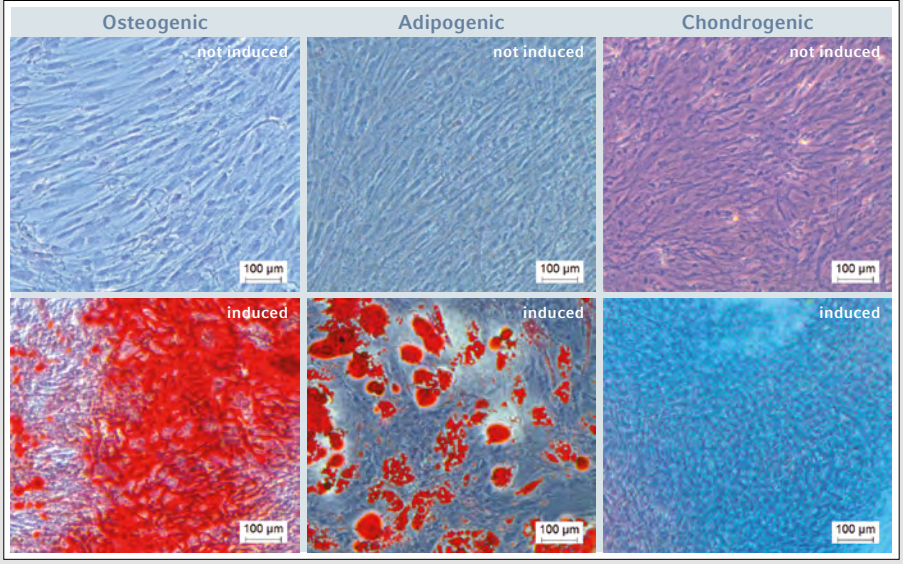

Fig. 3: Multi-lineage differentiation potential of hMSC-BM after long-term expansion on the CCCadvanced FN1 motifs surface in an animal-component-free environment

As suggested by positive specific staining shown in Fig. 3, after successive passages on the FN1 motifs surface hMSC-BM preserve a robust multipotency-associated differentiation potential. In parallel, the hMSC-specific immunophenotype was analysed by flow cytometry analysis (data not shown). Results confirm that even after long-term expansion on the FN1 motifs surface, hMSCs remain positive for the expression of mesenchymal markers and negative for hematopoietic lineage markers.

Conclusion

The ready-to-use CCCadvanced FN1 motifs surface from Eppendorf efficiently supports long-term hMSC-BM expansion in a completely defined, synthetic culture system. All through the expansion process, hMSCs maintain a stable and robust proliferation rate and typical hMSC morphology. Furthermore, after successive passages on the FN1 motifs surface in an ACF culture system, cells retain their typical marker expression profile as well as their multi-lineage mesodermal differentiation potential.

The suitability of the FN1 motifs surface to support efficient hMSC-BM proliferation in combination with different commercial XF culture media has been evaluated with success, facilitating the establishment of a defined environment for hMSC cultivation.

*Please refer to **www.eppendorf.com/appnote390** for detailed materials and methods.

Literature

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