

Process Parameters

Contents presented in this Supplementary Document are relative to the process parameters or quality attributes taken into account for the comparison of normoxic (i.e., 5% CO₂; 21% O₂) and hypoxic (i.e., 5% CO₂; 2% O₂) incubation conditions of hFPTs. Specific comparative evaluation results are presented for each parameter or attribute, for the incubation conditions investigated in this study. The objective was to assess if a switch from normoxic toward hypoxic incubation conditions may be considered for hFPT cultures, based on parametric and pondered evaluation results.

Table PP1. Definition of process parameters and quality attributes within the comparative hFPT manufacturing process. For each item, the predefined targets and the IPCs or PPCs to be appropriately monitored are listed, along with the corresponding acceptance criteria. Grading was performed for the comparative evaluation of incubation conditions using the abbreviated nomenclature presented hereafter. (−) = negative effect of hypoxic incubation, (+/−) = no impact of hypoxic incubation, (+) = positive effect of hypoxic incubation. CPPs were defined as parameters exerting a critical effect on the safety and quality of the final manufactured cell batch. KPPs were defined as parameters exerting a key effect on the quality of the final manufactured cell batch. CQAs were defined as attributes presenting critical implications in the safety and quality of the final manufactured cell batch. KQAs were defined as attributes presenting key implications in the quality of the final manufactured cell batch. CPP, critical process parameter; CQA, critical quality attribute; hFPT, human fetal progenitor tenocytes; IPC, in-process control; KQA, key quality attribute; KPP, key process parameter; PPC, post-process control.

CPP/CQA KPP/KQA	Targets	IPCs/PPCs	Acceptance criteria (cumulative)	Evaluation of hypoxia conditions vs. standard normoxia conditions
CPP/CQA Cellular morphology	Specific hFPT cellular morphology and behavior maintenance in hypoxic culture	Visual monitoring of hFPT cultures during expansion	Spindle-shaped cells	+/-
	Absence of observable multiple cell populations in hypoxic culture	Photographic recording of hFPT cultures	Distinctive fibroblastic phenotype Absence of multiple cell populations	
KPP/KQA Cellular proliferation rate	Maintenance or amelioration of hFPT proliferation rates in hypoxic culture	Evolutionary monitoring of population doubling values	Equal or superior population doubling values in hypoxia vs. normoxia	+
		Evolutionary monitoring of population doubling times	Equal or inferior population doubling times in hypoxia vs. normoxia	

KPP/KQA Cell size distribution	Maintenance of hFPT size distribution in hypoxic culture or relative shift of cell size distribution toward smaller cells	Visual monitoring of hFPT cultures during expansion Photographic recording of hFPT cultures Determination of cell size distribution by flow cytometry	Maintained cell size distribution or shift of cell size distribution toward smaller cells	+
CPP/CQA Cell surface markers	Maintenance of hFPT cell surface marker panel in hypoxic culture conditions	Determination of cell surface marker panel by flow cytometry	Maintenance of cell surface marker panel	+/-
CPP/CQA Phenotype plasticity under induction	Maintenance of low hFPT phenotype plasticity in hypoxic culture conditions	Determination of hFPT susceptibility toward chemical adipogenic and osteogenic induction	Maintenance of low cell phenotype plasticity Absence of increased susceptibility toward chemical adipogenic and osteogenic induction	+/-
KPP/KQA Protein contents	Maintenance of expression for characteristic hFPT protein components in hypoxic culture conditions Increase in expression of proteins with potential therapeutic functions in hypoxic culture conditions	Determination of protein expression and protein contents during and following cell expansion	Maintenance of expression for characteristic protein components (e.g., extracellular matrix components)	+

CPP/CQA	Absence of contaminants	No increased rates of contaminating pathogens detected in retention samples from hypoxic culture conditions	Monitoring of culture medium coloration (i.e., phenol red indicator) and turbidity	Absence of detection for specified contaminants or values of detection < to specified thresholds	+/-
			Specific testing for bacteria, fungi, viruses, endotoxins, mycoplasma	Absence of detection for non-specified contaminants	
CPP/CQA	Cellular viability	Maintenance of adequate cellular viability upon hFPT enzymatic harvest and upon initiation from cryopreservation	Determination of relative cellular viability following cell harvest or cell initiation	Relative cellular viability \geq 85% after harvest	+/-
				Relative cellular viability \geq 80% after initiation	
CPP/CQA	Cell adherence in culture	Adherence of \geq 70% of cells after the first 24 h of hypoxic incubation following hFPT cell seeding	Visual monitoring of cultures after 24 h of incubation	Cell adherence \geq 70% after 24 h of incubation	+/-
			Photographic recording of hFPT cultures		
KPP/KQA	Cell monolayer homogeneity	Homogenous growth of hFPT cell monolayer over available culture vessel surfaces in hypoxic culture conditions	Visual monitoring of cell monolayer during expansion	Absence of unpopulated culture surfaces \geq 15% of the total available culture surface in each vessel	+/-
			Photographic recording of cultures		
CPP/CQA	Cell population purity	Specific hFPT cellular morphology and behavior maintenance in hypoxic culture conditions	Visual monitoring of cultures during expansion	Spindle-shaped cells	+/-
			Photographic recording of cultures	Distinctive fibroblastic phenotype	
		Absence of multiple cell populations in culture	Determination of cell surface marker panel by flow cytometry	Absence of multiple cell populations Consistent cell surface marker panel	

KPP/KQA Cellular metabolism	No modification in hFPT cellular metabolism rate in hypoxic culture conditions	Monitoring of the culture medium coloration (i.e., phenol red indicator) and turbidity	No significant differences in culture medium color and turbidity	+/-
KPP/KQA Culture confluency evolution	Confirmation of positive hFPT cell monolayer confluency evolution between successive medium exchange procedures	Visual monitoring of confluency at medium exchange procedures Photographic recording of cultures	(Confluency value at medium exchange X) \geq (Confluency value at medium exchange X-1)	+/-
KPP/KQA Cell type lifespan	No modifications in hFPT cell type lifespan or increased cell type lifespan in hypoxic incubation conditions	In vitro cell type lifespan determination Evolutionary monitoring of cell population doubling values and doubling times	No modifications in cell type lifespan or increased cell type lifespan	+
KPP/KQA Total culture period	Conserved or shortened hFPT total culture period for specific in vitro expansions in hypoxic culture conditions	Evolutionary monitoring of cell culture periods, from seeding to harvest	Conserved or shortened total culture period for specific in vitro expansions	+