Understanding and leveraging cell metabolism to enhance mesenchymal stem cell transplantation survival in tissue engineering and regenerative medicine applications

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Abstract: In tissue engineering and regenerative medicine, stem cell-specifically, mesenchymal stromal/stem cells (MSCs)-therapies have fallen short of their initial promise and hype. The observed marginal, to no benefit, success in several applications has been attributed primarily to poor cell survival and engraftment at transplantation sites. MSCs have a metabolism that is flexible enough to enable them to fulfill their various cellular functions and remarkably sensitive to different cellular and environmental cues. At the transplantation sites, MSCs experience hostile environments devoid or, at the very least, severely depleted of oxygen and nutrients. The impact of this particular setting on MSC metabolism ultimately affects their survival and function. In order to develop the next generation of cell-delivery materials and methods, scientists must have a better understanding of the metabolic switches MSCs experience upon transplantation. By designing treatment strategies with cell metabolism in mind, scientists may improve survival and the overall therapeutic potential of MSCs. Here, we provide a comprehensive review of plausible metabolic switches in response to implantation and of the various strategies currently used to leverage MSC metabolism to improve stem cell-based therapeutics.

Significance statement: Lack of success of stem cell-based therapies has been largely attributed to the massive cell death observed post-transplantation, which is caused by the metabolic shock these cells experience as they transition from in vitro to a hostile, injured site in vivo. The metabolism in mesenchymal stem cells (MSCs), specifically, is highly sensitive to cellular and environmental cues. In order to improve cell survival rate post-transplantation, it is important that scientists understand, and take into account, the needs and demands of MSC metabolism as they design the next generation of MSC-based therapies.

1- Introduction:

Mesenchymal stromal/stem cells (MSCs), also referred to as ñtissue-specific skeletal stem cells,ò1 are a non-hematopoietic subpopulation of cells with multi-lineage potential to differentiate into various tissues of mesodermal origin.2, 3 MSCs possess a promising therapeutic potential for tissue engineering applications because of their ability to both differentiate into distinctive mesenchymal phenotypes and to induce a regenerative microenvironment by secreting bioactive chemical compounds that inhibit tissue scarring, inhibit apoptosis, narrow or contain the field of injury, promote angiogenesis, and stimulate mitosis of tissue-intrinsic stem or progenitor cells.4, 5 Nevertheless, stem cell-based therapeutics have fallen short of the initial promise and hype, resulting in inconsistent preclinical and clinical trial outcomes. Although several studies addressing various medical applications confirmed the efficacy of MSC therapy, other investigations reported no therapeutic benefits.6-10 In many instances, the effect of MSC-based therapies either decreased or disappeared over long-term outcome assessments11-13 or underperformed compared with conventional therapies and/or therapeutic expectations.14, 15

The shortcomings of stem cell-based therapeutics have been attributed primarily to poor cell survival and engraftment, with

cell survival being the crucial aspect. It is now well established that cells experience high mortality rate upon transplantation at the injury site; in fact, as low as 1% of MSCs survive 1 day after implantation.3, 16, 17 In material scaffolds, which are meant to provide the appropriate structure and environment for delivered cells to take effect, studies have also shown low survival rates, with as high as 90% MSC mortality by day 7 postectopic implantation18-20 and 85% by day 14 when implanted in a critical segmental femoral bone defect.21 For these reasons, the observed benefits of MSC therapy have been largely credited to the delivery of cell signals, or paracrine effects, instead of the direct differentiation of MSCs.22 Improved survival of transplanted MSCs, however, could increase direct contributions by cell differentiation, extracellular matrix deposition, cell-host-cell interactions, and/or, at the very least, longer term secretion of paracrine factors. Such outcomes could subsequently increase the overall therapeutic potential of MSCs, and in applications where the beneficial effect of MSCs is marginal to none, improved MSC survival may reverse previously unsuccessful clinical/therapeutic strategies.

Upon transplantation, cells experience a hostile, injured microenvironment of ischemia (lack of oxygen and nutrients),18 inflammation, and oxidative stress comprising superoxide anions and hydrogen peroxide.3, 23 This situation is further exacerbated by poor removal of cell metabolic waste due to the lack of functional vascular networks at the injury site. This adverse microenvironment encountered by the transplanted cells has direct effects on cell metabolism, which is the set of life-sustaining chemical reactions. Vital cell processes are powered through the metabolism of energy substrates supplied by the environment, such as glucose, fatty acids, and amino acids.24 Beyond survival of transplanted cells, tissue repair is an energy-demanding task; metabolism fuels organogenesis, including processes involved in directing stem cell fate, proliferation, and self-renewal.24 For these reasons, understanding and addressing the metabolic needs of MSCs may enhance their survival upon transplantation and, subsequently, their therapeutic effect.

Although various approaches aiming at enhancing cell survival existð including, but not limited to, gene-editing, novel biomaterials, growth-factor signaling, and so onð , the present review will focus on approaches that either directly or indirectly affect the metabolism of MSCs. Specifically, in this review, we will discuss metabolism in MSCs in different scenarios as well as state-of-the-art strategies that leverage cell metabolomics to improve cell survival after transplantation. We will not discuss the technical aspect or methodology for assessing MSC metabolism (for reference, see [25-27]), but rather, we will focus on the link between metabolism and cell survival.

2- MSC METABOLISM

2-1 Metabolic plasticity

Cell metabolism is the sum of all chemical reactions through which energy is created from breaking chemical bonds and used to synthesize basic components. These reactions are directly linked to the activity/function of cells; in fact, different activities and functions have different energy demands and require synthesis/removal of different molecules. In this regard, plasticity in energy metabolism (or bioenergetics) allows stem cells to match the divergent energy demands of self-renewal and lineage specification24 and, in the case of transplanted cells, of cell survival.

Figure 1A illustrates the basic bioenergetic reactions that take place in MSCs. Energy generated in cells is stored in energy carrier molecules called adenosine triphosphate (ATP), which can be used in future cell chemical reactions. In MSCs, ATP is produced by two pathways: glycolysis and oxidative phosphorylation (OXPHOS). In glycolysis, glucose is consumed to produce pyruvate and ATP; pyruvate is either converted into lactate or, in the presence of sufficient oxygen, oxidized in mitochondria to acetyl-CoA, which feeds into the tricarboxylic acid (TCA) or, Krebs, cycle. In OXPHOS, electron by-products of the TCA cycle pass through the electron transport chain (ETC) at the mitochondrial cristae and produce ATP molecules. Although OXPHOS averages a significantly higher ATP production than glycolysis (specifically, 34-36 vs 2 ATP molecules per glucose molecule24, 28), studies have consistently shown that MSC survival is not dependent on OXPHOS but rather on glycolysis. Under standard culture conditions, treatment of bone marrow-derived (BM) rat MSCs (rMSCs) with 2,4-dinitrophenol (DNP), a mitochondrial uncoupler that inhibits OXPHOS showed no increase in cell death up to 72 hours post-DNP treatment29, whereas inhibition of glycolysis by 2-deoxyglucose induced a significant decrease in cell viability as early as 24 hours after treatment in BM-rMSCs29 and BM-human MSCs (hMSCs).30 Moreover, Moya et al30 showed that, in near-anoxia (0.1% pO2), disrupting the TCA cycle using Antimycin A or Malanate (TCA cycle inhibitors) does not further impair the MSC metabolism nor does it significantly increase the cell mortality rate. Altogether, these findings confirm that only glycolysis is crucial for cell survival.

Beyond survival, glycolysis is also a hallmark of ñstemnessò and the preferred pathway for stem cell self-renewal/proliferation. Compared with OXPHOS, glycolysis is less efficient in generating energy; however, inefficient ATP production is a problem only when resources are scarce, which is not the case for proliferating mammalian cells in vivo.31 In fact, proliferating cells have important metabolic requirements that extend beyond ATP, and aerobic glycolysis meets both the bioenergetics and biosynthetic demands of producing new cells.28, 31 Aerobic glycolysis is able to fuel the anabolic intermediates needed for the synthesis of amino acids and nucleotides, via the pentose phosphate pathway (Figure 1A).28, 31, 32 Aerobic glycolysis is defined as the conversion of glucose to lactate even in the presence of sufficient oxygen to support glucose catabolism via the TCA cycle with OXPHOS.28 Vander Heiden et al33 approximated that, following glycolysis in proliferating cells, only 5% to 10% of the pyruvate is diverted to mitochondria, whereas 85% is converted to lactate, essentially diminishing the contributions from the TCA and OXPHOS pathways during cell proliferation (known as the Warburg Effect).

Similarly, when MSCs begin to differentiate, they switch their metabolic activity depending on the differentiation pathway they undertake. For example, BM-hMSCs cultured under chondrogenic conditions prefer glycolysis, as demonstrated by their reduced oxygen consumption and OXPHOS activity.34 In contrast, BM-hMSCs undergoing osteogenic differentiation exhibit a decrease in glycolytic enzymes but increases in respiratory enzymes, oxygen consumption, and mitochondrial biogenesis; these results suggest a bioenergetics switch from glycolysis to OXPHOS.35 hMSCs experience a similar switch to promote adipogenic differentiation.36 Because these experiments have been conducted in vitro, however, differentiation protocols may play a role in the observed metabolic changes.

Metabolic flexibility allows MSCs to perform their functions; however, it should be noted that metabolic pathways are highly sensitive to cellular and environmental cues, such as oxygen levels, availability of substrates, and pH levels. In order to develop better cell-based therapies and improve MSC survival and their therapeutic potential, scientists should consider the microenvironment in which the cells are transplanted and its potential effect on cellular metabolic activities that ultimately affect cell survival and function.

2.2 Effects of different oxygen tension levels

It is well established that oxygen tension has a direct effect on cellular energy metabolism and, thus, affects cell survival and their therapeutic potential. For this reason, it is important to understand the oxygen tension levels that cells will experience upon transplantation and their subsequent effects on cell metabolism. Oxygen tension varies greatly depending on the targeted tissue, as well as the state (healthy vs injured) of said tissue. Oxygen tension was shown to fall from 2.6% to 3.3% to 0.9% pO2 within the first 5 minutes of an arterial occlusion in rodent hearts37 and to reach near-anoxia (0.2% pO2) after 30 minutes of ischemia.38 In joint diseases, oxygenation levels of the synovial fluid are lower in inflammatory cases.39, 40 In situ measurements in the intervertebral disc, however, reported a minimum of 0.3% to0.7% pO2 in the disc center in both healthy canine41, 42 and degenerated human disks.43 In general, oxygen tension in various parts of the mammalian body is considerably lower in vivo than the atmospheric oxygen tension (21%) and ranges from 12% in the blood to as low as 1% in the deep zone of cartilage regions.44

Under standard cell culture conditions, MSCs are kept at 21% pO2, which is considered ñnormoxiaò but is not physiologically relevant (physioxia). Upon implantation, cells are affected by their new microenvironment, which triggers metabolic changes and signaling pathways. Overall, lack of oxygen triggers the ñswitchò from high-energy yielding processes (specifically, glycolysis combined with OXPHOS) to the low-energy yielding process of glycolysis (Figure 1B).44 In environments with pO2 between 1% and 3%, BM-hMSCs45 and BM-rMSCs46 further activate the Akt signaling pathway by phosphorylation. Akt facilitates metabolic reprogramming toward glycolysis and maintains cell proliferation when the oxygen supply is restricted.47 This signaling pathway plays a key role in the stabilization of the transcription factor hypoxia-inducible factor (HIF)-1.44

In the presence of ñsufficientò oxygen levels, the oxygen-regulated HIF-1Ŭ subunit is degraded by proteasomes.48, 49 Under hypoxic conditions, however, HIF-1Ŭ escapes oxygen-driven proteasomal degradation. Moya et al30 reported that exposure of BM-hMSCs to near-anoxia (specifically, 0.1% pO2) for 3 days resulted in significantly higher HIF-1Ŭ expression and bioactivity compared with results obtained when BM-hMSCs were maintained at 1%, 5%, and 21% pO2. Stabilized HIF-1Ŭ translocates to the cell nucleus, where it dimerizes with HIF-1ƀ (Figure 1B). The HIF-1Ŭƀƀƀƀ dimers activate the transcription of genes encoding glycolytic enzymes and glucose transporters.50, 51 These upregulated glycolytic enzymes aid in shunting energy metabolism from OXPHOS to glycolysis alone and in boosting glycolytic activity (Figure 1B).52, 53 Increased expression of glucose transporters further increases the influx of glucose molecules and overall glycolytic activity in cells.

It is worth noting that small differences in oxygen tension (eg, 1% vs 0.1%) result in a cascade of metabolic and signaling pathways in MSCs. For this reason, it is essential that studies report the level of oxygen tension as accurately as possible. Traditionally, control of pericellular oxygen tension is achieved by fixing the oxygen tension in the gas phase above the medium under which cells are cultured.54-56 Pericellular tension, however, also depends on the number of cells present and their metabolic rate as well as on the diffusion distance from the medium surface to the bottom of the cell culture plasticware where cells are attached.55 Pettersen et al55 showed that after 7 days under standard cell culture conditions (21% pO2) of human T47D cells, the pO2 was 17.63% at the top of the culture medium and only 7.51% at the bottom of the plasticware surface where the cells were cultured. In order to better simulate the in vivo milieu of the transplantation site in vitro, researchers should study the oxygen tension level that more closely resembles that of the targeted site in vivo.

2.3 Effects of energy substrate availability

Historically, the lack of oxygen had been regarded as the primary cause of cell death in an ischemic environment. Recent research, however, provided evidence that the lack of nutrients, notably glucose, is the main culprit of cell death postimplantation. Published reports by many groups have shown that BM-hMSCs,30, 57, 58 BM-rMSCs,29 and BM-sheep MSCs59 are able to survive in a hypoxic to near-anoxic environment in vitro, given that glucose is available. Glycolysis, which is inherently anaerobic, is the only production path for ATP in cases where little-to-no oxygen is present, which renders glucose the most important energy substrate for MSC survival. Moya et al30 confirmed experimentally that BM-hMSCs cultured under 0.1% pO2 are unable to use exogenous glutamine, serine, or pyruvate to produce ATP. The MSC mortality rate under each of the aforementioned conditions was approximately 93% by day 14 of cell culture in vitro.30 In contrast, BM-hMSCs supplied with glucose remained up to 60% viable under near-anoxia by day 14 of cell culture.30 Nevertheless, it should be noted that the high sensitivity of MSC survival to glucose deprivation

cannot be explained solely by the loss of ATP.29 It is now known that, beyond ATP depletion, inhibition of glycolysis due to glucose deprivation also leads to loss of essential prosurvival signals such as Akt.29, 60 Furthermore, even in the presence of oxygen, glucose deprivation or starvation, activates metabolic stresses, which may induce cell death.61-64 In this scenario, autophagy is activated as a protective mechanism to mitigate cell stress-induced damage and provide nutrients for short-term survival (Figure 2).61

In the face of limited or no access to nutrients, cells activate their nutrient-sensing mechanisms to switch from an anabolic program to a catabolic one (Figure 2). By decreasing their anabolic activities, cells diminish their metabolic demand, and by activating catabolic pathways, they cannibalize themselves in order to increase their metabolic supply. Cells accomplish this switch through two major cell signaling mechanisms: (i) inactivation of the target of the rapamycin (TOR) pathway (mTOR), a master regulator of protein translation and proliferation, and (ii) activation of the AMP-activated protein kinase (AMPK). These mutually antagonistic signaling pathways reduce energy-intensive processes (such as protein and lipid synthesis) and induce autophagy and lysosomal biogenesis in order to recover metabolites and energy from existing macromolecules and thus ensure cell survival.68, 69 A study by Nuschke et al58 reported that MSC autophagy was highly sensitive to glucose concentrations. BM-hMSCs that were transferred from a hyperglycemic (4.5 g/L) to a physiological level of glucose medium (1 g/L), thereby lowering their glucose supply, accumulated higher levels of LC3-II (an autophagy marker) as a function of time. In contrast, various oxygen tensions (specifically, 21%, 4%, and 1% pO2) did not have an effect on the accumulation, or lack, of autophagomes.58 Autophagy is a temporary response to the shortage of glucose, and cell death may still occur if the problem is not addressed in a timely manner.61 Therefore, regardless of the level of oxygen tension, the lack of glucose may lead to cell death.

To the best of our knowledge, there are no reported in vivo measurements of glucose concentrations in the heart, bone, or articular cartilage in the literature. Because glucose is paramount for the survival of MSCs, knowledge of this parameter is needed and should be used by scientists when designing delivery vehicles for MSCs for tissue engineering and regenerative medicine applications.

2.4 Effects of the mechanical microenvironment

Recent scientific studies provided evidence that the mechanical environment is an integral component of mammalian cell and tissue milieu and should also be taken into consideration regarding cell metabolism. For example, dynamic compression was shown to increase ATP and lactate production, as well as glucose consumption of pig intervertebral disc cells,70, 71 and to enhance the glycolytic energy flux in human chondrocytes.72 Mechanical stress was also shown to facilitate the transport of glucose into rat podocytes by increasing the number of glucose transporters on the surface of these cells, thus altering energy production and metabolism.73 Although MSCs are highly mechanosensitive, as shown in many studies regarding stem cell fate in response to mechanical stimulation,74-76 there are no literature reports correlating MSC mechanosensitivity and metabolism.

2.5 Effects of pH

In addition to the effects on oxygen and nutrient levels, the lack of vascular network results in the accumulation of lactic acid (the end product of glycolysis under conditions of insufficient oxygen), which acidifies the local environment (Figure 1B). Although less detrimental than glucose shortage, lower intracellular and extracellular pH levels inhibit MSC metabolism, proliferation, and osteogenic differentiation.77-83 Acidifying the extracellular medium of rabbit MSCs to pH 6.8-7.0 resulted in large decreases in the glucose consumption rate and the lactate production rate (48.3% and 95.1%, respectively), compared with standard cell culture conditions at pH >7.4.78 This finding suggests a reduction in glycolysis, which may be a feedback system to avoid further acidification of the extracellular space that would ultimately lead to an excessive acidification of the cytosol and cell death.77, 78 Moreover, Massa et al81 showed that extracellular acidosis (specifically, pH 6.5) induces the quiescence state in BM-hMSCs, which may partially explain the observed reduction in cell proliferation and differentiation potential under acidosis.

In conclusion, the effects of oxygen tension levels, glucose supply, mechanical stress, and pH levels so far reported in the literature regarding MSC metabolism highlight and reiterate the importance of these conditions in the microenvironment that MSCs encounter upon transplantation in vivo. Unfortunately, there is limited pertinent information in the literature about the in vivo conditions at different transplantation sites and their impact on cell metabolism. Furthermore, research on MSC metabolism has been largely restricted to standard cell culture practices, which are not analogous to either the transplantation milieu or other in vivo scenarios. For all the aforementioned reasons, there is a crucial need to bridge this knowledge gap in order to elucidate the processes of MSC metabolism and to improve MSC survival and, ultimately, MSC-based therapeutics.

3- STRATEGIES FOR ENHANCING MSC SURVIVAL POSTIMPLANTATION

In tissue engineering and regenerative medicine applications, strategies to mitigate cell death take precedence since increased cell survival is hypothesized to enhance the effectiveness of cell-based therapies. It is imperative that cells maintain at least a minimum energy status in order to carry out life-sustaining metabolic reactions and, therefore, survive. In order to maintain the necessary energy status in the compromised metabolic milieu of transplanted sites, scientists have pursued two main strategies: (i) providing cells with the substrates necessary in order to maintain their bioenergetics levels and (ii) modulating the metabolism in MSCs to promote cell adjustment to their new environment.

3.1 Providing MSCs with nutrients and oxygen

In the design of cell-containing scaffolds for tissue engineering, the scaffold architecture should be optimized to enable the mass transport of oxygen and vital nutrients necessary for the energy requirements of the transplanted cells. Because upon implantation, cell-containing scaffolds and the surrounding tissue are avascular, there is no convective mass transport. Consequently, the grafted cells rely mainly on the availability of nutrients, whose diffusion coefficients into the 3D material scaffolds are generally relatively low. In this context, the structural features of the scaffold are critical for optimal mass transport conditions.84 Pore size, geometry, orientation, interconnectivity and branching, and channels may all affect diffusion. Because tissue regeneration and engineering specifications vary with specific tissue requirements, there is no consensus regarding the optimal structural parameters of material scaffolds. Recently, new technologies, such as additive manufacturing, or 3D printing, enable production of scaffolds with customable macroscopic porous networks that were not possible before.

Nevertheless, focusing on designing scaffolds that improve mass transport of nutrients may not be the ideal or, at least, a sufficient approach considering that MSC-containing scaffolds are often implanted in ischemic environments and, thus, require extracellular nutrients to promote a regenerative response to the injured tissue. Consequently, research should refocus its effort to rationally engineer a new generation of cell-containing scaffolds that provide nutrients tailored to the needs of transplanted cells. Given that glucose (and not oxygen) is essential to ensure MSC survival via glycolysis,29, 30, 57 recent strategies have focused on delivering this chemical compound to cells. Reports by our research group have provided evidence that glucose-loaded scaffolds result in an increased expression of HIF-1Ŭ by BM-hMSCs and in a fivefold enhancement of BM-hMSC survival rate postimplantation in an ectopic mouse model at day 14.57

In addition to short-term survival, it is essential that engineered scaffolds deliver the amount of glucose necessary to meet at least the minimum MSC energy requirements until newly formed vessels reach a density within the scaffold sufficient to provide cells with the needed nutrients. The goal is to aid transplanted cells survive until vascularization takes place. After all, in order to contribute to tissue regeneration via cell proliferation and differentiation, cells cannot be in either a nutrient- or an oxygen-deprived environment; their bioenergetics should not operate at the minimum level. To this end, glucose delivery is a challenging endeavor because this molecule, which is uncharged and highly water-soluble, is rapidly released from scaffolds. Furthermore, increasing the concentration of glucose within scaffolds is not a viable strategy since glucose at high concentrations can disturb the osmotic pressure and cause cell lysis.85

These challenges motivated our research group to develop an enzyme-controlled glucose-delivery hydrogel that uses starch, similar to that present in plants, to store large amounts of glucose while reducing the associated osmotic pressure.86 In this novel system, the starch, which is osmotically inactive, is enzymatically converted to glucose by amyloglucosidase. Preliminary results provided evidence that such scaffolds enhanced MSC survival in vitro and in vivo (ectopic mouse model) for up to 14 days.87 However, further investigations are still needed to determine whether, in this scenario, the buildup of lactate (due to glycolysis under conditions of insufficient oxygen) and the possible subsequent drop in pH levels could become a limiting factor in tissue engineering constructs of clinically meaningful size.

Other research groups have proposed oxygen delivery systems, including oxygen-carrier scaffolds and oxygen-producing scaffolds.88-90 The rationale of these studies was that oxygen is required to ensure MSC survival. However, based on recent data, which provide strong evidence that MSCs rely on glycolysis to survive in near-anoxia,29, 30, 59 the authors of this review propose another hypothesis; specifically, that supplying oxygen to MSCs enables flexibility in energetic substrate choice because oxygen-dependent energetic pathways become functional under these conditions. An additional positive effect of delivering oxygen to transplanted MSCs is that oxygen is a critical regulator in maintaining the stemness of MSCs and in determining their differentiation fate (review reference [91]).

Although oxygen delivery systems have been largely explored, there are few studies concerning MSCs directly. Namely, Newland et al90 showed that co-culturing oxygen-producing microspheres with hMSCs at near-anoxia (0.1% pO2), with 0.05 mg/mL of glucose (contained in the fetal bovine serum), maintained cell viability up to day 4 in vitro. In a similar strategy, oxygen-loaded microspheres were embedded within polyprolactone constructs (termed oxygen microtanks) in order to enhance their oxygen delivery capability. In vitro coculture of oxygen microtanks with adipose-derived hMSCs at 0% pO2 also maintained cell viability up to day 4, then drastically dropping off to approximately 20% viability at day 6.89 In vivo, the use of synthetic oxygen-carrier-enriched hydrogels for delivering murine mesenchymal stem-like C3H10T1/2 cells resulted in increased cell survival in an ectopic mouse model at day 3 but not at day 7.92 In this study, delivery of oxygen did not increase blood vessel volume density, number, or thickness.92 A possible explanation for this outcome is that delivery of oxygen may have temporarily ñmisledò transplanted cells, so that they did not reorient their paracrine functions appropriately toward an angiogenic response. Consistent with this hypothesis, MC3T3-E1 cells cultured on oxygen-releasing hollow particles exhibited a significant downregulation of both HIF-1Ŭ and VEGF when compared with cells cultured on hollow particles alone at 1% pO2.89 In contrast, conditioned medium from hMSCs exposed to near-anoxia led to a twofold increase in chemotaxis of human umbilical vein endothelial cells and to a threefold increase in the formation of vascular structures when compared with the results obtained using conditioned medium from hMSCs cultured at either 21% or 5% pO2.93 Taken together, these results provide evidence that, although delivering oxygen may enhance cell survival, it may also hinder angiogenesis and, thus, affect regeneration in some tissues. Nevertheless, further research is needed to provide mechanistic insights into the relationship among oxygen delivery, MSC survival, and angiogenesis, and to establish the importance of oxygen delivery as a strategy to improve cell survival post-transplantation. Oxygen delivery systems must be reassessed using MSCs, because different cell

types require distinctly different energetic and biosynthetic pathways to support their specific functional needs.

Last but not least, the field has become increasingly invested in developing vascularization techniques in order to enable in situ nutrient supply and cell metabolic waste removal by convective transport. Unfortunately, induced vessel ingrowth into the cell-containing scaffolds is often too slow to provide adequate, timely nutrient transport and availability to the cells in the core of 3D transplanted constructs. To overcome this limitation, newly developed strategies include material scaffold design, inclusion of pro-angiogenic components (such as angiogenic growth factors or seeded endothelial cells), and in vitro pre-vascularization. For more comprehensive information about these developments, the reader is referred to the following reviews.94-96

3.2 Modulating MSC metabolism

Keeping in mind that transplanted MSCs encounter environments lacking nutrients and other chemical requirements for their bioenergetics needs, several research groups have turned to modulating MSC metabolism in order to lower their bioenergetics-related demand. For example, a recent strategy has consisted in driving MSCs to a reduced metabolic state before their implantation in animal models.67 To this end, cells are induced to quiescence (by serum deprivation), which is a state that stem cells physiologically adopt in their biological niche in order to preserve their key functions. This quiescence preconditioning allowed BM-hMSCs to withstand exposure to total glucose depletion under continuous near-anoxia (0.1% pO2) for up to 14 days67 and under hypoxia (2% pO2) for up to 75 days in vitro.97 In vivo, quiescence preconditioning significantly improved BM-hMSC viability when ectopically implanted in cell-containing constructs for up to 7 days.67 More importantly, upon in vitro reperfusion, preconditioned BM-hMSCs maintained both their proliferation and secretory functions, as well as their trilineage differentiation potential.67, 97

From a metabolic perspective, quiescent BM-hMSCs exhibited reduced ATP-consuming anabolic functions, such as nucleotide and protein syntheses, but maintained their intracellular ATP and protein contents,67 suggesting that quiescence may have redirected the energy metabolism of BM-hMSCs toward essential ñhousekeepingò functions.98 Furthermore, quiescence preconditioning stimulated autophagic activity in BM-MSCs, as measured by the sustained inhibition of mTOR throughout the early (3-day) period of ischemia exposure in vitro.67 Overall, our research group hypothesizes that, through reprogramming toward a reduced metabolic state, quiescence preconditioning provides a protective adaptation of hMSCs against abrupt transition to the deleterious ischemic environment.

Strategies have also been developed by other research groups to precondition cells prior to transplantation to a hostile in vivo environment, thus enhancing their survival and functional performance. Because MSC-based therapies are delivered to ischemic/hypoxic injured sites, it was suggested that a hypoxic treatment could precondition the cells to adapt better to the ischemic environment. In fact, increasing numbers of literature reports have shown that hypoxic preconditioning of MSCs, under various oxygen concentrations and periods of time, enhances in vitro cell viability and in vivo cell engraftment.99-109 For example, Beegle et al99 demonstrated that hypoxia-preconditioned BM-hMSCs had an approximately twofold increase in survival at 6 days under serum deprivation and hypoxia (1% pO2) as compared with control BM-hMSCs. Preconditioning cells for 96 hours at 1% pO2 reduced glucose consumption and lactate secretion in MSCs99 and regulated their glycogen metabolism through phosphoinositide 3-kinase/AKT and HIF-1/glycogen synthase kinase-3ß-mediated pathways, thus producing glycogen-based energy prestorage.51 Furthermore, Stegen et al110 reported that genetic or pharmacological HIF-1Ŭ stabilization (which occurs under hypoxia) in skeletal progenitor cells prior to implantation improved survival of these cells by adapting glutamine and glycogen metabolism to preserve redox and metabolic energy balance, resulting in enhanced bone repair.

Apart from ischemia, the site of injured tissue is usually associated with oxidative stress, inflammation, and acute immune response107; therefore, other preconditioning and treatment strategies such as exposure to oxidative stress and heat shock treatment have been explored for MSCs.111, 112 Although no direct survival studies have been carried out, results showed an enhancement of the therapeutic potential of such preconditioned MSCs.112 For a deeper discussion of these preconditioning strategies, the readers are referred to the following reviews.107, 113

4- CONCLUSION

The metabolic activity of MSCs, which is highly sensitive to environmental and intracellular signaling cues, affects the survival, function, and fate of these cells postimplantation. In order to improve the survival of transplanted MSCs and, thus, potentially improve the efficacy of stem cell therapies, it is crucial to bridge the gap in current knowledge regarding MSC metabolism in a clinically relevant microenvironment. To date, research endeavors have already redefined previous misconceptions and clearly attributed a paramount role to glucose, instead of oxygen, in the survival of MSCs in an ischemic milieu such as the one these cells encounter postimplantation at injured sites.

Considering the importance of metabolism to all key functions of cells, the new generation of cell-containing scaffolds should be rationally designed taking into account cell metabolic mechanisms and needs. Oxygen tension levels, nutrient starvation and glucose depletion, various pH levels, and so on, are parameters that drastically differ between standard cell culture practices and transplantation sites, and they have critical effects on the metabolic activities of cells and, thus, on their survival and function in the post-transplantation milieu. Recent strategies for improving the survival rate of transplanted MSCs have focused on providing these cells with substrates necessary to sustain bioenergetic levels, as well as modulating their metabolism so that these cells better adjust to their new hostile environment. Ultimately, leveraging metabolism as a strategy to enhance cell survival needs further investigation. Scientists should seek to ameliorate the metabolic transition of transplanted MSCs from cell culture

practices in vitro to the demanding transplantation milieu in vivo in order to improve their survival and, subsequently, their functions pertinent to tissue repair and formation.

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CONFLICT OF INTEREST

D.L.-A. declared Technology intellectual property rights with Transfer Accelerator Office. H.P declared patent ownership of ñTime-controlled glucose releasing hydrogels and applications thereof EP3011952A1ò. The other authors indicated no financial relationships.

AUTHOR CONTRIBUTIONS

G.E.S.-N., G.L., C.D., E.P., D.L.-A.: conception and design, manuscript writing; M.P., A.M., M.B., R.B.: manuscript writing; H.P.: conception and design, manuscript writing, final approval of the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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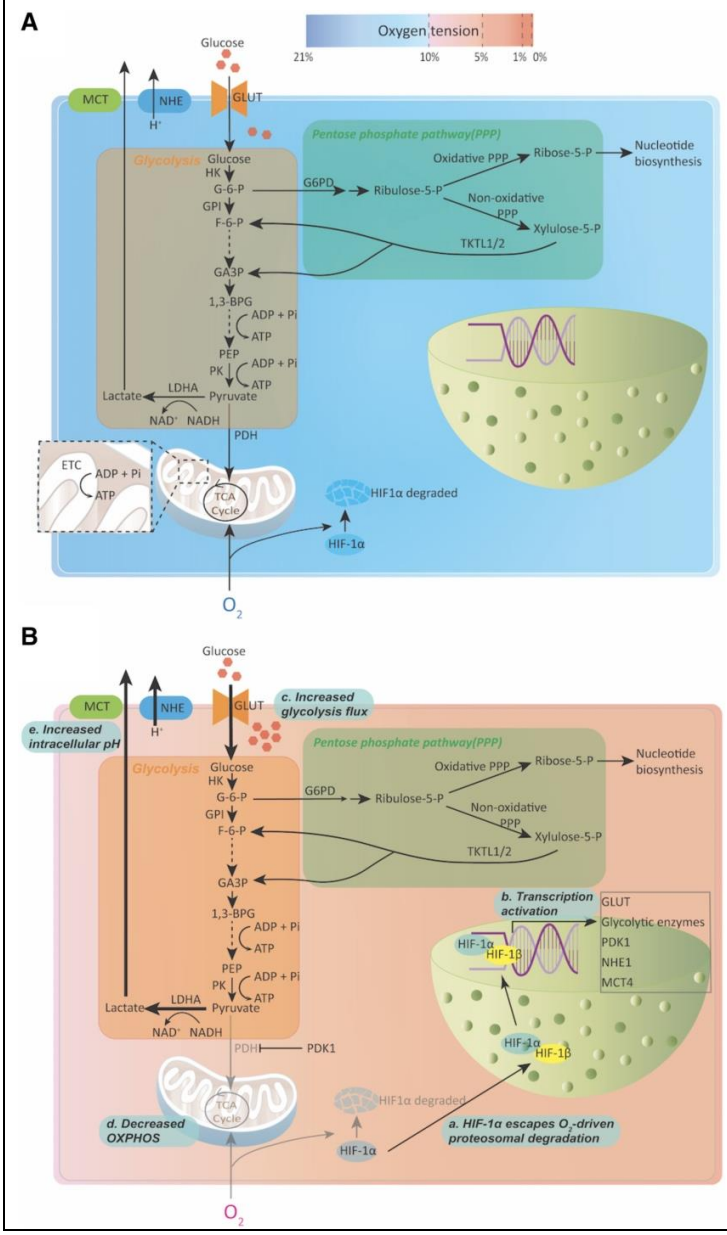

Figure 1:

Schematic diagrams illustrate metabolism in mesenchymal stem cells (MSCs) under different oxygen tension levels. A, Under "sufficient" oxygen, glucose molecules are broken down in glycolysis for the production of ATP molecules. By-products of glycolysis and other chemical components enter the tricarboxylic acid (TCA) cycle in mitochondria. In OXPHOS, electron by-products of the TCA cycle pass through the electron transport chain (ETC) at the mitochondrial cristae and produce more ATP molecules. Moreover, HIF-1α is degraded under "sufficient" oxygen-level conditions by proteosomes. B, In a near-anoxia environment (pO2 < 0.1%), however, (A) HIF-1α escapes oxygen-driven proteosomal degradation and translocates to the nucleus, where it dimerizes with HIF-1β. Accumulation of HIF-1 α/β activates (B) the transcription of genes that increase expression of glucose transporters and glycolytic enzymes, which (c) increase glycolytic flux; and (d) decrease activity and eventually block the OXPHOS pathway. Increased activity of the glycolytic pathway yields a buildup of lactate, which (e) increases intracellular pH. 1,3BPG, 1,3-bisphosphoglycerate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate; GA3P, glyceraldehyde-3-phosphate; GLUT, glucose transporter; GPI, glucose-6-phosphate isomerase; HIF, hypoxia-inducible factor; HK, hexokinase; LDHA, lactate dehydrogenase; MCT, Monocarboxylate transporter; NHE, Na+-H+-exchanger; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase; PEP, phospho-enol-pyruvate; Pi, inorganic phosphate; PK, pyruvate kinase; PPP, pentose phosphate pathway; Ribose-5-P, ribose-5-phosphate; Ribulose-5-P, ribulose-5-phosphate; TKTL1/2, transketolase1/2; Xylulose-5-P, xylulose-5-phosphate

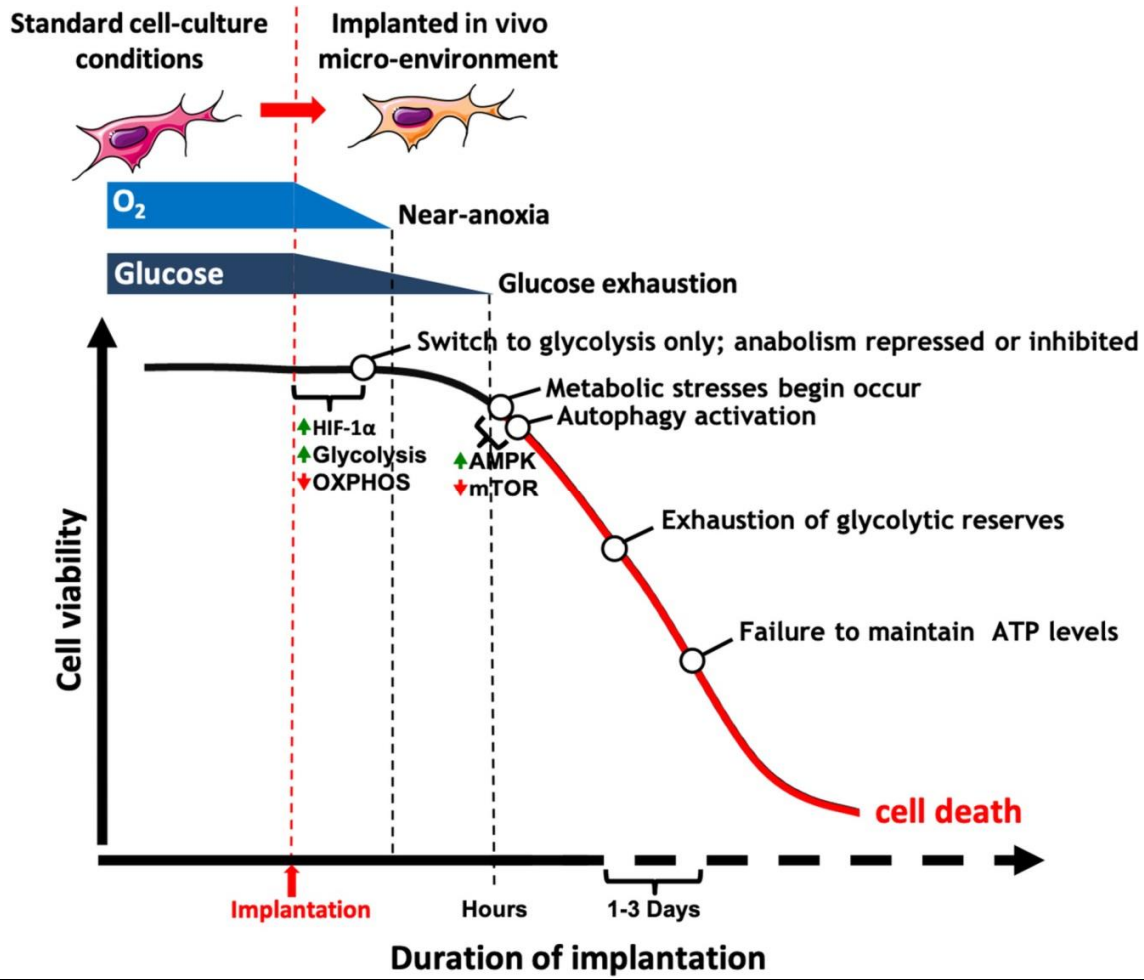

Figure 2: Schematic illustrates the envisioned timeline of bioenergetic metabolic activity of cells upon implantation. Before implantation, mesenchymal stem cells (MSCs) are generally cultured under standard conditions of 21% pO2 and with "sufficient" glucose. Upon implantation in an ischemic site, MSCs experience insufficient levels of oxygen tension, which triggers a switch to glycolysis only and represses, eventually inhibiting, anabolic activities. Upon glucose exhaustion, MSCs begin to experience metabolic stresses, which are, in part, counteracted by the activation of autophagy. Autophagy, or self-catabolism, reduces sources of stress and provides nutrients during times of nutrient withdrawal. Eventually, the glycolytic reserves are exhausted, and autophagy is unable to either provide MSCs additional cell nutrients or mitigate cell stress. Ultimately, cell death occurs when cells do not meet their minimal bioenergetic demands. The time points indicated are according to literature reports (30, 65-67) and may vary according to different culture conditions, such as cell density and mass transport parameters. This figure was modified from the graphical abstract from Moya et al 30