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Age associated communication between cells and matrix: a potential impact on stem cell-based tissue regeneration strategies

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A recent paper demonstrated that decellularized extracellular matrix (DECM) deposited by synovium-derived stem cells (SDSCs), especially from fetal donors, could rejuvenate human adult SDSCs in both proliferation and chondrogenic potential, in which expanded cells and corresponding culture substrate (such as DECM) were found to share a mutual reaction in both elasticity and protein profiles (see ref. [1]). It seems that young DECM may assist in the development of culture strategies that optimize proliferation and maintain "stemness" of mesenchymal stem cells (MSCs), helping to overcome one of the primary difficulties in MSC-based regenerative therapies. In this paper, the effects of age on the proliferative capacity and differentiation potential of MSCs are reviewed, along with the ability of DECM from young cells to rejuvenate old cells. In an effort to highlight some of the potential molecular mechanisms responsible for this phenomenon, we discuss age-related changes to extracellular matrix (ECM)'s physical properties and chemical composition.

Keywords: mesenchymal stem cells, aging, extracellular matrix, tissue engineering, proliferation, differentiation, microenvironment

Abbreviations and Acronyms: ACAN, aggrecan; ALP, alkaline phosphatase; ADSC, adipose derived mesenchymal stem cell; BMSC, bone marrow derived mesenchymal stem cell; CBFA1, core binding factor α 1; CFU-OB, colony forming unit of osteoblasts; COL2A1, collagen type 2 alpha1; DECM, decellularized extracellular matrix; ECM, extracellular matrix; ESC, embryonic stem cell; FGF2, fibroblast growth factor basic; GAG, glycosaminoglycan; HGF, hepatocyte growth factor; HSC, haematopoietic stem cell; IGF-I, insulin-like growth factor I; LOXL1, lysyl oxidase-like 1; LPL, lipopolysaccharide; LV, left ventricle; miRNA, micro-RNA; MMP, matrix metalloproteinase; mRNA, mRNA; MSC, mesenchymal stem cell; ON, osteonectin; PPARG, peroxisome proliferator active receptor gamma; ROS, reactive oxygen species; RUNX2, runt-related transcription factor 2; SD, Sprague-Dawley; SDSC, synovium derived stem cell; SIS-ECM, small intestinal submucosa extracellular matrix; SOX9, SRY (sex determining region-Y)-box 9; SPARC, secreted protein, acidic and rich in cysteine; TGFβ, transforming growth factor β; TIMP, tissue inhibitor of metalloproteinases; UDSC, umbilical cord derived mesenchymal stem cell; VEGF, vascular endothelial growth factor.

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Introduction

Multipotent and present in many tissues, mesenchymal stem cells (MSCs) can be extracted, grown *in vitro*, differentiated into a variety of cell types, and subsequently implanted in the original donor without risk of immunological rejection. These properties give MSCs great potential for the treatment of degenerative diseases. Significant resources have been devoted to developing effective MSC-based therapies. Since degenerative diseases primarily affect the

aged, it is important to appreciate the changes that the MSC population undergoes with aging, and to develop techniques to reverse age-associated handicaps to the MSC population.[2]

A comprehensive discussion of MSC aging ought to address intrinsic aging, defined by Sharpless as changes located within the stem cell and its progeny, as well as extrinsic differences between young and old MSCs.[3,4,5] For brevity's sake, this review will focus on extrinsic aging. Important intrinsic changes, including but not limited to micro RNA (miRNA) expression,[6,7] telomere length,[8,9] expression of apoptotic proteins and cell cycle regulators,[7,10] cellular secretome (reviewed in refs.[11,12]), transmembrane receptors,[13] and general differences in gene expression[14] have been reviewed elsewhere.[15,16]

Relative to intrinsic changes, the extrinsic changes that accompany cellular aging have been sparsely studied, which is unfortunate given the close relationship between the extracellular matrix (ECM) that makes up the MSC microenvironment and cellular behavior.[17,18] It has been suggested that MSCs from different mouse strains (SAMP6, SAMR1, C57BL/6) with different life expectancies may be intrinsically similar in their *in vitro* proliferation and differentiation capacity, despite exhibiting dramatically different *in vivo* differentiation and proliferation capacity due to the influence of heterogeneous microenvironments.[19] Furthermore, age is associated with changes in the ECM that have been linked to multiple pathologies (reviewed in ref.[20]), including cancer.[17] Consequently, it is vital that the impact of ECM aging on MSC behavior needs to be addressed in order to better understand age-associated diseases and MSC-based regenerative therapy. This review aims to succinctly discuss the current understanding of how ECM ages and to highlight the impact this process has on MSC proliferation and differentiation (**Fig. 1**).

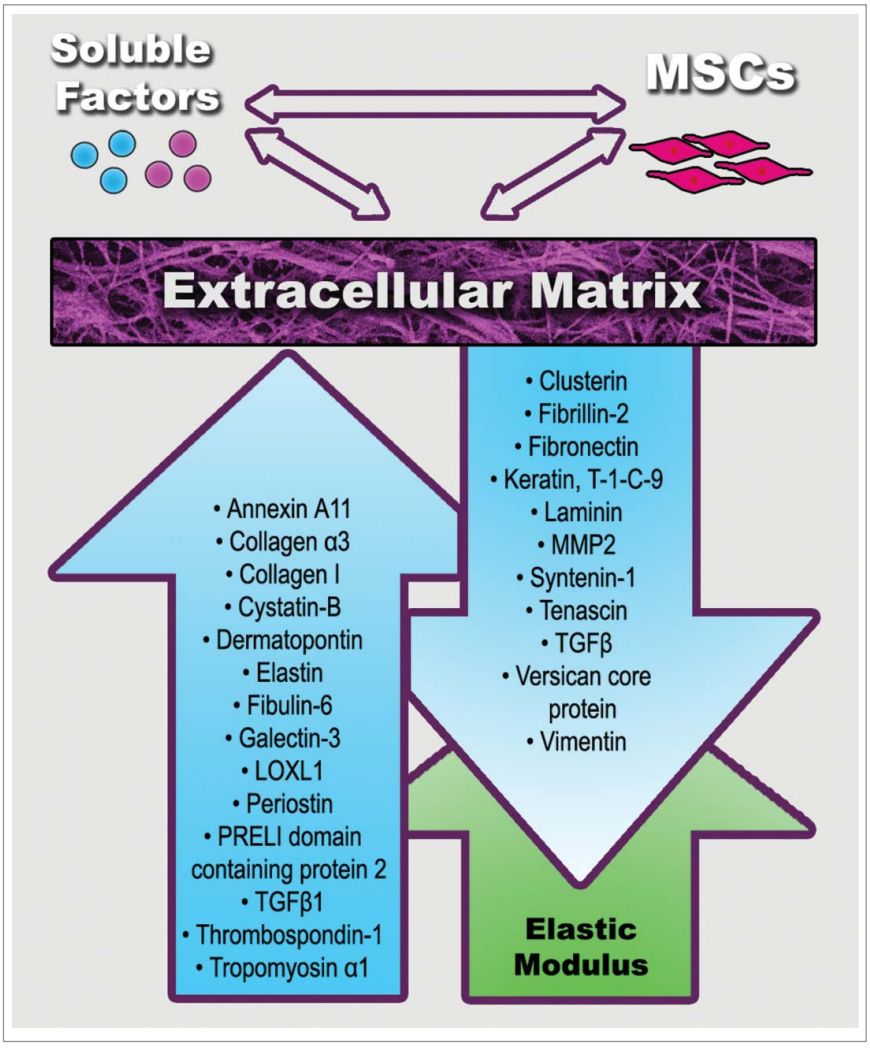

Figure 1. Physical (green color) and compositional (blue color) age-related changes in extracellular matrix (ECM) formed by mesenchymal stem cells (MSCs). Up/down arrows indicate increase or decrease. See **Tables 1 and 2** for references.

Donor Age Dependent Cell Senescence

Aging affects MSC proliferative capacity

Like many of the body's cells, MSCs change with age (reviewed in ref.[15]). Aging is associated with depressed proliferation and elevated apoptosis of MSCs. A recent report compared the self-renewal ability in murine (female C57BL/6 mice) bone marrow derived MSCs (BMSCs) from 3-month-old and 18-month-old mice. Three-month-old BMSCs generated 5 times the number of colony forming unit of osteoblasts (CFU-OB) after expansion, divided by a fraction of cells used for expansion, on plastic culture.[21] Kretlow et al. found that murine BMSCs from younger animals had significantly elevated proliferation rates.[22] It was further found that BMSCs from Wistar rats aged < 1 month old had a doubling time of 26.07 ± 1.81 hours and a doubling number of 3.64 ± 0.19 while rats aged > 12 months old had a doubling time of 32.20 ± 3.89 hours and a doubling number of 3.07 ± 0.18, suggesting that the young BMSCs replicated more quickly and to a greater degree than did the old BMSCs.[23] This phenomenon was also observed in rhesus macaques where BMSCs from young monkeys had more rapid proliferation rates than those from older monkeys.[6]

The above animal studies have counterparts in human tissue research. Zhang and coworkers showed that human fetal BMSCs had a higher proliferative rate than adult adipose derived MSCs (ADSCs) and umbilical cord derived

MSCs (UDSCs).[24] It was observed by Stenderup and colleagues that BMSCs from young donors (18–29 y old) had greater proliferative capacity (41 ± 10 versus 24 ± 11 population doublings), slower progression to senescence, and greater proliferative rate (0.09 ± 0.02 vs. 0.05 ± 0.02 population doublings/day) than BMSCs from old donors (68–81 y old).[25] Mareschi and coworkers contrasted BMSCs from pediatric donors with young adult donors and reported that, after 112 d of culture, BMSCs from pediatric donors had a cumulative population density almost double that of BMSCs from young adult donors (10.2 ± 1.9 versus 5.5 ± 3.7),[26] suggesting that pediatric BMSCs have increased proliferative capacity *in vitro*. Similarly, Zaim et al. compared the proliferation of human BMSCs from young (0–12 y old), adult (25–60 y old), and elderly (over 60 y old) donors and reported that young BMSCs had a greater proliferative lifespan than cells from the other donor groups (38 ± 8 versus 30 ± 6 versus 10 ± 6 population doublings, respectively).[27] Fickert observed that human BMSCs from donors younger than 50 y old or older than 65 y old had increased proliferation rates relative to donors between 50–65 y old (but donors older than 50 had a wider range in doubling times).[28] Another publication reported that expression of apoptosis markers was higher in aged (older than 40 y old) human BMSCs than in young (younger than 19 y old) and adult (19–40 y old) human BMSCs and that, after 5 weeks of culture, proliferation in the aged BMSC cultures declined relative to BMSCs from adult donors.[29]

Aging may affect MSC differentiation, but reports conflict

There have also been many conflicting reports on whether age causes changes in MSC population size and differentiation capacity.[30] For example, Asumda et al. reported that BMSCs from young Sprague-Dawley (SD) rats (4 months old) had greater adipogenic, chondrogenic, and osteogenic differentiation potential than BMSCs from old rats (15 months old).[31] Similarly, Kretlow et al. reported that murine BMSCs had decreased chondrogenic and osteogenic differentiation capacity with age across all test groups (6 day, 6 week, 1 year), but that adipogenic differentiation ability declined only in cells from the oldest animals.[22] Wilson and colleagues observed a progressive decline in osteogenic capacity with age in BMSCs taken from C57BL/6WT mice.[32] In humans, bone marrow cells isolated from males between 37–80 y old exhibited an age dependent decline in alkaline phosphatase (ALP) activity (a marker of osteogenesis) and transcript number in osteogenic culture conditions, suggesting a diminished osteogenic capacity of BMSCs with age.[33] This finding differs from results reported by Stenderup and coworkers, who found that human BMSCs from young donors (18–29 y old) and old donors (68–81 y old) had similar osteogenic and adipogenic capacity,[25] as well as the results of Fickert et al., which did not reveal any difference in osteogenic differentiation between BMSCs harvested from humans younger than 50 y old or between 50–65 y old.[28] However, the work of Zaim et al. illustrated that adipogenic, osteogenic, and neurogenic differentiation potential of human BMSCs declined with age, but that chondrogenic potential did not.[27] Zhang reported that, following exposure to osteogenic induction medium, human fetal BMSCs exhibited greater osteogenic differentiation than adult ADSCs and UDSCs. Interestingly, they further found that the scaffold constructed by fetal BMSCs demonstrated elevated expression of osteogenic genes [runt-related transcription factor 2 (*RUNX2*), collagen type I (*COL1A1*), osteonectin (*ON*), and *ALP*], relative to the scaffolds formed by UDSCs or ADSCs.[24] Kanawa and colleagues, however, reported that the adipogenic and osteogenic differentiation capacities of human BMSCs were unchanged by age, but that BMSCs' chondrogenic capacity declined.[34]

As has been reviewed elsewhere,[35] molecular differences between young and aged MSCs likely partially account for the phenotypic differences between youthful and aged stem cells. Approximately 8000 genes were differentially expressed between murine (C57BL/6 WT) BMSCs from 2-, 8-, and 26-month-old sources. However, only 86 genes were downregulated across the entire 2–26 month time period studied; among these genes were osteogenic markers (*ALP*) and growth factors [vascular endothelial growth factor (*VEGF*), hepatocyte growth factor (*HGF*), and insulin-like growth factor I (*IGFI*)].[32] Interestingly, Wilson and coworkers further reported that, in BMSCs of mice, adipogenic markers increased between 2–8 months old but declined between 8–26 months old.[32] It was reported that expression of Nanog, an embryonic stem cell (ESC) marker, gradually declined as source animal age increased in BALB/c mice BMSCs,[36] and that, in human BMSCs from donors aged 15–79 y old, expression of core binding factor α 1 (*CBFA1*), an osteogenic transcriptional factor, decreased with age (though the difference was not statistically significant). Meanwhile, expression of peroxisome proliferator active receptor gamma (*PPARG*), an adipogenic transcription factor, increased nearly fourfold and SRY (sex determining region Y)-box 9(*SOX9*, the master regulator of chondrogenesis) was unchanged.[37] Kasper et al. studied the changes in SD rat BMSC proteomes associated with aging, and attributed the inverse correlation between age and replicative potential in part to a decline in cellular responsiveness to mechanical stimuli resulting from a less dynamic actin cytoskeleton.[38]

Veronesi and coworkers reviewed studies on aging's effects on ADSCs and BMSCs, and concluded that, although some authors found no differences in the proliferative capacity of MSCs, the majority of studies agreed that decreases in MSC proliferation rate and osteogenic capacity were observed with age.[16] Some have postulated that inter-laboratory differences in MSC isolation and culture may partially account for conflicting reports on MSC proliferation rate,[39] and others have raised the possibility that differences in murine strain might also be responsible, since inter-strain differences exist in murine haematopoietic stem cells (HSCs).[30] The differentiation capacity and proliferative ability of MSCs *in vitro* is likely to correlate with their regenerative capacity *in vivo*,[21,31] thus the diminished proliferative capacity of aged MSCs must be addressed before MSC-based therapies can be optimized for the treatment of degenerative diseases.

Rejuvenation of Elderly Cells by Young ECM

It has been proven that culture on decellularized ECM (DECM) substantially elevates MSC proliferative capacity relative to culture on plastic. Our laboratory has repeatedly demonstrated the superior ability of DECM, relative to plastic, to enhance MSC proliferation and chondrogenic potential (reviewed in ref.[40]) and other researchers have reported similar findings. For example, it was found that the proliferative ability of MSCs on plastic could be elevated up to 250-fold by culture on basement membrane ECM proteins.[41] Enhanced proliferation and differentiation capacities were observed in both murine BMSCs[42] and human BMSCs[41] following culture on marrow cell produced DECM relative to plastic. For a more thorough review of the role of DECM in preventing senescence of cultured MSCs, see refs.[2,40]

While DECM culture in general is superior to culture on plastic, there is evidence to suggest that the properties of DECM and its efficacy in *in vitro* culture systems is highly influenced by the chronological age of the cells that formed it. Work by Conboy and colleagues showed that joining the circulatory systems of old (C57B1/6) and young (2–3 months old) mice (C57Bi/Ka-Ly5.2) elevated *in vivo* hepatocyte proliferation and enhanced *in vivo* repair of muscle damage in old (19–26 months old) mice, while also stimulating both *in vitro* and *in vivo* proliferation of aged satellite cells (myocyte precursors).[42] Interestingly, Yu and colleagues reported that, in rhesus macaque BMSCs, conditioned medium obtained from young (1–5 y old) BMSCs was unable to elevate the proliferation rate of old (12–20 y old) BMSCs.[6] This finding suggests that the factors secreted by young stem cells alone are unable to elevate the proliferation rates of old stem cells which, as will be discussed below, is not true of DECM formed by young stem cells.[1] The combination of these reports highlights both the ability of the stem cell niche to regulate stem cell behavior and the importance of ECM as a component of that niche.

The ECM appears to convey to cells signals that regulate their proliferation and maintain "stemness."[40,43] Whether directly, through its own physical properties, or indirectly, though sequestration or concentration of soluble factors, the ECM plays a major role in regulating the activities of nearby cells.[17,44] A comparison of decellularized organ scaffolds found that fetal and juvenile (3 months old to 1.5 y old) rhesus monkey kidney DECM allowed greater organ repopulation and tubular structure formation than adult (5–13 y old) DECM.[45] Choi and colleagues found that DECM produced by young cells restored a youthful phenotype to senescent human fibroblasts, resulting in an additional 25 population doublings (a 39% increase in cellular lifespan).[46] This finding was applied to female mouse (C57BL/6) BMSCs by Sun and colleagues, who reported that the defective replication of aged (18 months old) BMSCs was reversed by exposure to DECM from young (3 months old) animals. They found that cells from both young and old mice had a higher expression of telomerase when cultured on DECM from young donors than when cultured on DECM from aged donors or plastic, and that the young DECM diminished reactive oxygen species (ROS) levels in aged BMSCs by 50%.[21]

Our own laboratory has shown that expansion on DECM deposited by fetal synovium-derived stem cells (SDSCs) enhanced the proliferation of human adult SDSCs to a greater extent than plastic or DECM deposited by adult SDSCs. Further, fetal DECM diminished human adult SDSC ROS levels and promoted apoptotic resistance relative to plastic culture.[1] Our findings are similar to the recent work of Ng and colleagues, who found that human adult BMSCs cultured on human fetal DECM had, after 10 d of culture, 1.6 times greater cell population size than BMSCs on plastic. Further, BMSCs that were cultured on plastic for 6 passages and then moved to fetal DECM displayed a 2.2-fold higher cell count after 3 additional passages relative to BMSCs cultured on plastic throughout, suggesting that fetal DECM was able to rescue the aged phenotype of adult BMSCs.[47] Taken together, these studies show that DECM formed by young cells is able to independently enhance the proliferation of older cells. As mentioned above, this finding suggests a possible utility of fetal DECM in generating sufficiently large MSC populations derived from elderly donors for use in regenerative therapies.

There is also evidence to suggest that a young ECM may not only rejuvenate the replicative capacity of old MSCs, but also their capacity to form tissues. Pre-culture of old murine BMSCs on DECM formed by young BMSCs resulted in greater *in vivo* bone formation by old BMSCs than by old BMSCs pre-cultured on old DECM or plastic.[21] Kurtz et al. observed increased expression of pluripotency markers and differentiation potential in old MSCs following seeding on DECM formed by young ADSCs.[48] Our work showed that, after incubation in chondrogenic or adipogenic medium, human adult SDSCs expanded on fetal DECM had greater production of chondrogenic marker genes [*SOX9*, aggrecan (*ACAN*), and collagen type II (*COL2A1*)], glycosaminoglycan (GAG) and collagen type II, or adipogenic markers lipopolysaccharide (*LPL*) and *PPARG*, respectively, though this pattern was not observed following treatment with osteogenic medium.[1] These studies very strongly suggest that culture of old MSCs on young DECM rejuvenates the ability of old MSCs to differentiate.

Physical Changes of Matrix with Age and Potential Influence on MSC Commitment

General changes in matrix physical properties

Some researchers have noted that the stiffness of the ECM in muscle increases with age in animals[49,50] and that an increase in ECM stiffness may enhance integrin signaling and cell proliferation.[51,52] The effects of this change on aging satellite cells have been reviewed.[53] It has long been established that collagen crosslinking increases as ECM ages as a result of the Maillard reaction which is believed to increase the stiffness of the matrix.[54] Tottey and colleagues studied the small intestinal submucosa ECM (SIS-ECM) harvested from 3-, 12-, 26-, and >52 -week-old porcine. They

Table 1. Age-associated changes in ECM physical properties. Matrix proteins related to aging are upregulated (Direct)

Correlation with age	Tissue source	Reference
GENERAL CHANGES		
Elastic modulus, rigidity, stiffness		
Direct	Porcine jejunum (3w, 12w, 26w, >52w)	55
Direct	Male Wistar rats, soleus muscle (4m, 24m)	50
Direct	Cardiac tissue of SD rats (fetal, neonatal, adult)	56
Direct (stiffness)	Epimysium of male Lewis rats (4 m, 28-30m)	49
Linear strength		
Direct	Porcine jejunum (3w, 12w, 26w, >52 w)	55
Thickness		
Direct	Porcine jejunum (3w, 12w, 26w, >52w)	55
MSC SPECIFIC CHANGES		
Elastic modulus		
Direct	Human synovium	1

reported that the ECM thickened with age and withstood less uniaxial stress. They also found that the elastic modulus of SIS-ECM from 3-week-old porcine was less than that of older animals, though the difference in SIS-ECM between 3 weeks old and >52 weeks old did not achieve statistical significance.[55] In rat cardiac tissue, it was also observed that DECM stiffness of neonatal (P2–3) hearts was double that of fetal hearts, but this trend did not continue between neonatal and adult (2–3 months old) SD rats.[56]

Interestingly, age also correlated with the stretching and partial unfolding of fibronectin in ECM, resulting in gradually increasing ECM tension, which might augment the effects of elevated collagen crosslinking.[57] The findings may partially account for Erickson et al.'s observation that the compressive modulus of adult (2–3 y old) and juvenile (3–6 months old) bovine cartilage was 50–75% greater than that of fetal (2nd or 3rd trimester) bovine cartilage.[58] These changes in matrix elasticity are important because ECM elasticity has been shown to affect cell proliferation,[59,60] although it is important to remember that the effects of matrix elasticity on MSC proliferation rates are sensitive to other factors, such as cell seeding density.[61] In **Table 1**, age-associated changes in the physical properties of ECM are summarized.

MSC specific changes in ECM physical properties

Cells on ECM interact with the matrix and sense the physical properties of the tissue that surrounds them,[62,63] a process that affects cell proliferation and apoptosis.[64,65] As discussed above, multiple studies have established that ECM changes with age, and therefore it stands to reason that age-related changes in the physical properties of ECM may affect MSC behavior. Matrix mechanical properties have been shown to affect stem cell proliferation rates[66] and lineage commitment,[67–70] likely by Rho GTPase dependent signaling.[71] It may therefore be expected that age-induced changes in the mechanical properties of ECM can affect MSC proliferation and differentiation potential. Unfortunately, the physical properties of MSC deposited ECM are usually studied in the context of differentiation and proliferation rates only; the potential differences between old and young ECM are relatively unexplored.

Li et al. showed that DECM deposited by human adult SDSCs was more elastic than that deposited by fetal SDSCs,[1] a finding in accord with the general findings of Tottey and coworkers.[55] Interestingly, Gershlak and colleagues cultured rat BMSCs on DECM isolated from cardiac tissue of fetal, neonatal, and adult SD rats, and reported that rat BMSCs generated higher traction force when cultured on a fetal DECM hydrogel mixture with a stiffness of 48 kPa, than on adult or neonatal DECM mixtures of that same stiffness.[56] Furthermore, the Badylak group, who grafted porcine SIS-ECM into the abdominal walls of adult female rats, observed that the SIS-ECM grafts taken from 3-week-old porcine withstood greater uniaxial tensile stress than those taken from 12-, 26-, and >52 -week-old porcine, as well as murine controls.[72] Other investigators found that seeding on a collagen type I and fibronectin coated polyacrylamide gel with an elasticity of 250 Pa (similar to bone marrow and adipose tissue), caused BMSCs to halt progression through the cell cycle, but that seeding on stiffer substrates caused non-proliferative BMSCs to enter the cell cycle.[66] These findings, viewed along with earlier reports that uniaxial tensile strength increases with age, suggests that the role of ECM in regulating MSCs is dependent not only on the physical properties of ECM, but on its chemical composition as well.[56] Age-associated changes in the make-up of ECM are therefore reviewed below.

Compositional Changes with Age and Potential Influence on MSC Commitment

General changes in ECM composition

Like its mechanical properties, the protein composition of ECM changes with age; however, these changes are often tissue specific. Magnuson and colleagues noted that fibronectin, a key component of ECM, undergoes changes in alternative splicing with aging both *in vitro* and *in vivo,* but that these changes are tissue specific.[73] Furthermore, it has been reported that the collagen content of human tissue increases with age in the left ventricle (LV) and uterine cervix,[74] but decreases in spinal discs.[75] Consequently, we divide our review of age-associated compositional modifications to the ECM by tissue type.

In muscle, the collagen content of the LV increased with age in wild-type mice,[76] sheep,[77] and humans (independent of pathology),[78–80] This change in LV collagen concentration may result from a variety of molecular factors recently reviewed.[81] Additionally, older (20 months old) BALB/c mice had more LV hydroxyproline, collagen, fibronectin, α-1 integrin, and α-5 integrin, but less β-1 integrin, than young (2 months old) or middle-aged (12 months old) mice.[82] Secreted protein, acidic and rich in cysteine (SPARC) increased with age in the LV

of sheep[77] and wild-type mice,[76] an interesting finding in light of SPARC's proposed role in sequestering procollagen from the cell surface and processing it into mature collagen fibrils.[76] As rat hearts matured from fetal to adult, there were significant increases in collagen types I and III and laminin.[56] Lindsey et al. showed that matrix metalloproteinase 3 (MMP3), MMP8, MMP9, MMP12, and MMP14 increased with increasing age in murine hearts,[83] though others observed a 40–45% decline in MMP2 activity in aged rat hearts.[84] Kostrominova and Brooks observed an age-associated decrease in mRNA (mRNA) coding for collagen types I, III, and V, elastin, and proteoglycan 4 in murine tendons.[85] It was found that age correlated directly with increased MMP2, MMP7, tissue inhibitor of metalloproteinase 1 (TIMP-1), TIMP-2, and TIMP-4 while MMP9 concentration decreased with age.[86] mRNA levels of *MMP2* and *MMP9*, as well as MMP2 and MMP9 activity, increased with age in tendons.[87]

Cartilage undergoes extensive modifications with age.[88] In brief, as discussed by Gentilli and Cancedda in their review of cartilage and ECM, fetal chondrogenesis entails a net synthesis of cartilage matrix, while normal adult cartilage requires a balance between growth and anabolism.[89] The total proteoglycan and collagen contents of the annulus fibrosus and nucleus pulposus decreased with age in humans, though some ECM proteins, such as fibromodulin and biglycan, increased in specific sections of the annulus fibrosus.[75] It has also been reported that aged bovine chondrocytes secreted approximately 60% less collagen than fetal bovine chondrocytes.[90] Erickson and colleagues showed that, without transforming growth factor β 3 (TGFβ3) treatment, the fetal bovine chondrocyte pellets had more collagen than juvenile or adult pellets.[58] In humans, the cartilage-like ECM produced by immature human chondrocytes was superior to that formed by adult chondrocytes.[91] Other researchers measured the ratio of collagen type III to collagen type I, and reported that age-related changes to this ratio were highly tissue specific in male Lewis rats; in cardiac tissues, the proportion of collagen type III increased from 1 day old to 6 month old rats, but fell between 1 y and 2 y. In the lungs, the proportion of collagen type III increased relatively steadily from birth until 2 years; in skin, the proportion of collagen type III decreased between 2 weeks and 1 month, but was constant after 2 months of age.[92]

Robert and colleagues thoroughly reviewed changes to the ECM with age and wrote that, in fibroblasts, fibronectin synthesis increased and collagen type III increased relative to collagen type I, while hyaluronan and GAG secretion decreased.[20] In bovine cartilage, GAG content was unaffected by age.[58] Proteoglycan and TGFβ1 decreased with age in the synovial fluid of New Zealand white rabbit knee joints.[93] Takubo et al. found that the hydroxyproline content of whole murine lung was higher at 24 months of age than at 3 or 6 months of age in BALB/c mice.[94] Senescent cells were shown to increase production of collagenases and to downregulate fibronectin and collagen types I, III, and IV,[95] which can be expected to weaken the ECM. The overall ECM biosynthesis seems to decrease with age, though this is not true of all ECM components.[20]

MSC specific changes in ECM composition

Stem cells are not exempt from compositional modifications to the ECM with age and these modifications likely impact their behavior.[96] Uncertainty exists regarding age-related changes in collagen synthesis by MSCs. Sun and colleagues found no difference in total protein content of ECM laid by young or old murine BMSCs, but did report that proteins weighing approximately 140 and 40 kDa were significantly less abundant in old ECM than in young.[21] Ng et al., however, found that the amount of ECM produced per cell by human fetal BMSCs and adult BMSCs was 1.4 ± 0.6 and 0.5 ± 0.2 μg, respectively, indicating that ECM production by fetal BMSCs is significantly greater than that by adult BMSCs.[47] BMSCs from elderly (1 y old) SD rats exhibited a diminished ability to generate a chondrogenic matrix *in vitro* relative to immature (1 week old) and young adult (12 weeks old) rats.[97]

Interestingly, Erickson and colleagues reported that bovine fetal BMSCs produced 2–15 times more GAG (in response to TGFβ3) and collagen than adult or juvenile BMSCs,[58] and it was noted by our laboratory that human adult SDSC expansion on fetal DECM yielded a greater GAG content per pellet, as well as a higher GAG/DNA ratio, than did expansion on adult DECM or plastic.[1] Sicari and colleagues found that the ECM produced by fetal porcine jejunum was enriched in GAG[72] and other researchers reported diminished GAG concentration in ECM with aging of murine lungs,[98] glomerular basement membranes, and cultured fibroblasts.[20] Tottey et al. found that 3-week-old porcine jejunum ECM had less fibroblast growth factor basic (FGF2)/mg dry weight than 12-, 26-, and >52 -week-old sources and less VEGF than 12- and 26-week-old sources; ECM from 3- and 12-week-old sources had greater sulfated GAG/mg dry weight than 26- or >52 -week-old sources.[55]

Changes of GAG concentration in ECM could have important implications for stem cell proliferation, as GAG levels influenced male adult Wister rat MSC lineage commitment[99] and proliferation.[100] Investigators have also found that aggrecan and collagen type II were highly expressed by one-week-old rat BMSCs, but diminished with age, as did collagen type IV, which helps assemble collagen type II fibrils. *LINK PROTEIN* and *SOX9* had increased expression in 12-week-old BMSCs compared to one-week-old or one-year-old BMSCs.[97] Our laboratory identified several proteins unique to fetal DECM, such as fibrillin-2, tenascin, versican core proteins, and clusterin, while adult DECM, by contrast, had more abundant dermatopontin, elastin, fibulin-6, periostin, thrombospondin-1, and TGFβ1.[1] In addition to its ability to interact with cells directly, ECM regulates the spatial distribution and availability of soluble factors that influence cell behavior.[17,43,101] For instance, previous work demonstrated that a deficiency in the proteoglycans biglycan and decorin resulted in elevated TGFβ and apoptosis rates in murine BMSCs[102] and GAGs were able to regulate murine bone formation by binding growth factors.[103]

Table 2. Age-associated changes in ECM composition. Matrix proteins related to aging are either up- (Direct) or downregulated (Indirect)

Protein	Correlation with age	Description	Tissue source	Reference
GENERAL CHANGES				
Collagens I and III	Direct (lung, heart) Indirect (skin)	Fraction of collagen III/(I+III), increased with age in lung and heart but decreased in skin	Male Lewis rat	92
Collagens I and III	Direct	Ratio of collagen III/I increased in fibroblast cultures	Human skin	108
Fibronectin	Direct	Plasma	Human	109
GAG	Indirect	Fibroblast culture	Human	111
Hyaluronic acid	Indirect	Skin	Human	110
Laminin	Direct	Glomerular Basement Membrane	Rat	20
Proteoglycans	Indirect	Glomerular Basement Membrane	Rat	20
MSC SPECIFIC CHANGES				
Annexin A11	Direct	Young (P3) versus Senescent (P12) ADSC	Human	48
Clusterin	Indirect	Adult versus Fetal DECM from SDSCs	Human	1
Collagen alpha-3(V)	Direct	Young (P3) versus Senescent (P12) ADSC	Human	48
Collagen I	Direct	Young (P3) versus Senescent (P12) ADSC	Human	48
Cystatin-B	Direct	Young (P3) versus Senescent (P12) ADSC	Human	48
Dermatopontin	Direct	Adult versus Fetal DECM from SDSCs	Human	1
Elastin	Direct	Adult versus Fetal DECM from SDSCs	Human	1
Fibrillin-2	Indirect	Adult versus Fetal DECM from SDSCs	Human	1
Fibulin-6	Direct	Adult versus Fetal DECM from SDSCs	Human	1
Fibronectin	Indirect	Young (P3) versus Senescent (P12) ADSC	Human	48
Galectin-3	Direct	Young (P3) versus Senescent (P12) ADSC	Human	48
Keratin, type 1 cytoskeletal 9	Indirect	Young (P3) versus Senescent (P12) ADSC	Human	48
Laminin	Indirect	Young (P3) versus Senescent (P12) ADSC	Human	48
LOXL1	Direct	Young (P3) versus Senescent (P12) ADSC	Human	48
Periostin	Direct	Adult versus Fetal DECM from SDSCs	Human	1
PRELI domain containing protein 2	Direct	Young (P3) versus Senescent (P12) ADSC	Human	48
MMP2	Indirect	Young (P3) versus Senescent (P12) ADSC	Human	48
Syntenin-1	Indirect	Young (P3) versus Senescent (P12) ADSC	Human	48
Tenascin	Indirect	Adult versus Fetal DECM from SDSCs	Human	1
TGFβ	Indirect	Young (P3) versus Senescent (P12) ADSC	Human	48
TGFβ1	Direct	Adult versus Fetal DECM from SDSCs	Human	1
Thrombospondin-1	Direct	Adult versus Fetal DECM from SDSCs	Human	1
Tropomyosin alpha 1 chain	Direct	Young (P3) versus Senescent (P12) ADSC	Human	48
Versican core protein	Indirect	Adult versus Fetal DECM from SDSCs	Human	1
Vimentin	Indirect	Young (P3) versus Senescent (P12) ADSC	Human	48

Seck and colleagues showed that age diminished the bone matrix content of IGF-I and IGF-II in men and IGF-I in women. As IGF-I and IGF-II are both important regulators of osteoblastic differentiation and proliferation, it is possible that these changes may affect BMSCs, despite the fact that no correlation was observed between these growth factors and bone remodeling.[104] Several other investigators have investigated the effect of age on the amount of growth factors sequestered within the matrix (reviewed in ref.[30]). It therefore seems clear that the compositional make-up of ECM changes with age and it is possible that these changes affect MSC proliferation both directly and indirectly by regulating the availability of growth factors. **Table 2** summarizes age-related compositional changes in ECM.

Conclusions and Future Directions

We have reviewed age-associated changes in MSC proliferative capacity and differentiation ability, as well as the use of DECM from young cells to rejuvenate old cells. Further, we have briefly explored some of the reported differences between the mechanical and chemical properties of old and young ECM. In general, it seems that the stiffness of ECM increases with age, and collagen and GAG concentrations, as well as the concentrations of several other protein components of ECM, may change with age in a highly source-specific fashion.

The seminal study of Engler et al. demonstrated that artificial matrices influence MSC differentiation toward the tissue type whose mechanical properties most closely mimic the synthetic matrix,[69] a finding that has been borne out repeatedly.[66,99,105] Studies by our laboratory have found that SDSCs became less susceptible to osteogenic or adipogenic differentiation following culture on DECM deposited by SDSCs.[106] Although this effect may be influenced by cell seeding density,[61] it seems plausible to posit, alongside others,[101] that *in vivo* ECM is optimized to encourage MSC differentiation toward their tissue of origin.

Our laboratory demonstrated that young ECM promotes MSC proliferation to a greater extent than plastic or even adult ECM. It is therefore tempting to speculate that fetal ECM may be optimized to promote the proliferation of stem cells in a manner similar to how adult ECM can direct MSC lineage choice. This hypothesis may explain, in

part, the many reports (discussed above) that younger MSCs exhibit greater *in vitro* proliferative capacity than aged MSCs. It seems intuitively self-evident that fetal stem cells possess a greater proliferative capacity than their aged counterparts, and indeed, UDSCs have elevated proliferation capacity relative to human adult BMSCs.[107] The many differences between fetal and adult ECM, therefore, might hold clues that will help researchers design culture techniques that optimize MSC proliferation and "stemness" maintenance *in vitro*, thereby removing one of the great obstacles to MSC-based regenerative medicine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Author Contributions

K.L.: collection and assembly of data, data analysis and interpretation, and manuscript writing; M.P.: conception and design, administrative support, manuscript writing, and final approval of the manuscript.

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