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Mechanics and Mechanobiology of Mesenchymal Stem Cell-Based Engineered Cartilage

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Overview

The intent of this manuscript is to review recent advances in the use of mesenchymal stem cells (MSCs) for the engineering of functional cartilage replacement tissues. Mesenchymal stem cells are a multipotent cell type capable of differentiating toward a number of lineages of the musculoskeletal system, including bone, cartilage and fat (Baksh et al. 2004). This multipotential capacity was first described over three decades ago (Friedenstein et al. 1974), and since then, the potential use of MSCs for regenerative therapies has generated tremendous excitement and focus. The attractiveness of MSCs for tissue repair is self-evident: in addition to their ability to take on multiple phenotypes, MSCs are readily expandable in culture and retain their multipotential characteristics with expansion; further, MSCs and other similar progenitor cells can be isolated from a wide variety of tissue sources, thereby avoiding further damage to diseased/injured tissues.

In this review, we outline seminal and recent work highlighting the potential of these unique cells in producing cartilage-like tissue equivalents. Specific focus is placed on the mechanical properties of engineered MSC-based cartilage and how these properties relate to that of engineered cartilage based on primary chondrocytes and to native tissue properties. We discuss current limitations and/or concerns that must be addressed for the clinical realization of MSC-based cartilage therapeutics, and provide some insight into potential underpinnings for the observed deviations from chondrocyte-based engineered constructs. We posit that these differences reveal specific deficits in terms of our description of chondrogenesis, and suggest that new benchmarks must be developed towards this end. Further, we describe the growing body of literature on the mechanobiology of MSC-based cartilage, highlighting positive findings with regards to the furtherance of the chondrogenic phenotype. We likewise discuss the failure of early successes to translate directly into engineered constructs with improved

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mechanical properties. Finally, we highlight recent work from our group and others that may point to new strategies for enhancing the formation of engineered cartilage based on MSCs.

Mechanical Properties of MSC-Based Engineered Constructs

As noted above, MSCs are a particularly ideal cell source for cartilage tissue engineering. Since the early descriptions of chondrogenesis in 3D cell pellet culture (Prockop 1997; Johnstone et al. 1998; Pittenger et al. 1999), the ability of these cells to generate cartilage-like tissues has been widely investigated. Indeed, a number of studies have demonstrated that, when presented with a chemically defined media including TGF/BMP family members, MSC chondrogenesis occurs in scaffolding materials previously employed for chondrocyte-based cartilage tissue engineering (Table 1). However, viability of MSCs in these materials is sometimes less robust than with chondrocytes (Salinas et al. 2007). Further material modifications, including covalent linking of matrix adhesion moieties (e.g., RGD and collagen mimetic peptides) can modulate both the viability of MSCs in these materials, as well as their phenotypic conversion (Connelly et al. 2007; Lee et al. 2008). In most studies, the shift of MSCs towards the chondrogenic phenotype is demonstrated via the induction of a relatively small set of phenotypic markers. Most commonly, these include expression of major extracellular matrix (ECM) components specific to hyaline cartilage, including aggrecan and type II collagen. These molecules play a central role in the establishment of the mechanical function of the native tissue. However, a host of other less prevalent matrix elements (including COMP, link protein, collagen type IX to name but a few) also play critical roles in matrix organization and retention, and are occasionally monitored during chondrogenesis as well. These ‘minor’ ECM components engender significant deficits in cartilage function and/or durability when absent from the tissue in disease or knockout animal models. In general, expression of these cartilage markers can reach levels consistent with chondrocytes, with matrix deposition throughout the material validated through immunohistochemical techniques (Figure 1).

While these markers are appropriate evidence that a chondrogenic event has occurred, they do not necessarily correlate with mechanical function. For clinical translation, and demonstration of efficacy, the mechanical properties of engineered constructs will ultimately dictate in vivo success. However, few studies of MSC chondrogenesis have considered this critical metric. Those few studies that have measured mechanical properties suggest a puzzling scenario. That is, while most markers of the ‘chondocyte’ phenotype are expressed (in some cases, to a greater extent than chondrocytes) the mechanical properties of constructs populated by these cells are typically inferior to both native tissue and to comparable tissue engineered constructs formed by fully differentiated chondrocytes. Where available, the mechanical properties of engineered MSC-based cartilage are provided in Table 1.

While somewhat limited in number, these studies do show that the the compressive properties of MSC-based constructs increase with culture duration, and do so in a number of materials, including hydrogels, non-woven meshes, and porous foams (Awad et al. 2004). For example, Figure 2 shows the time course of accumulation of mechanical and biochemical properties of constructs formed from bovine MSCs embedded within agarose, self-assembling peptide, and photocrosslinked hyaluronic acid hydrogels. These properties increase substantially over the 8 weeks of in vitro culture, with robust deposition of proteoglycan evident in histological sections (Erickson et al. 2009). More recent studies have shown that human MSCs in a natural ECM-derived material can reach a compressive modulus of ~0.15 MPa, and that bovine MSCs in agarose can reach a modulus of ~0.2 MPa with proteoglycan contents approaching 2–3% of the wet weight (Cheng et al. 2008; Huang et al. 2009). These values are significant when viewed in the context of chondrocyte-based tissue engineering efforts using serum containing media, but are deficient when compared to the properties of chondrocyte-based constructs cultured identically in pro-chondrogenic chemically defined, growth factor supplemented media. For

example, chondrocytes in agarose with transient exposure to TGF- β 3 regularly achieve native tissue levels of compressive moduli (on the order of 0.7–1.0 MPa) and proteoglycan content (5–6%) (Byers et al. 2006; Lima et al. 2007). Further, while compressive properties have been the predominant measure of mechanical function, other critical mechanical properties have also been investigated. For example, tensile properties are comparable between chondrocytes and MSCs in common 3D culture systems, though the final properties accrued remain far below native values (with correspondingly low collagen content) (Huang et al. 2008). These data support the growing appreciation that the functional capacity of MSCs has yet to be fully realized. Through the remainder of this review we attempt to understand the provenance of some of these limitations, and to suggest new thinking on methods to overcome these limitations.

Inhomogeneity and Inefficient Differentiation as Limiters of Functional Chondrogenesis

Given that the mechanical properties achieved to date with MSC-based constructs are poor relative to both the native tissue and chondrocyte-based constructs, specific factors must be identified and overcome to improve the functional capacities of these cells. Factors that may contribute to these functional limitations include heterogeneity in the starting cell populations and/or inefficiency of matrix deposition.

In terms of inhomogeneity of MSC populations, the trouble first arises in the lack of specific surface markers for identification of an MSC. While some markers have been postulated (CD105 and STRO-1 for example (Simmons et al. 1991; Majumdar et al. 2001)), the most common practice for MSC selection is through plastic adherence and colony formation. As a result, the selected population may include a heterogeneous mixture of MSCs and other cell types (Vogel et al. 2003), which may lack the differentiation capacity of MSCs, or limit the extent of MSC chondrogenesis. In one recent study, bone marrow was aspirated from the femoral canal of patients with osteoarthritis and 20 clonal populations were derived (Mareddy et al. 2007). Those clonal populations that proliferated rapidly were characterized tripotential (could form fat, bone, and cartilage phenotypes), whereas slow growing populations were typically unipotent or bipotent with varying combinations of osteogenic, chondrogenic, and adipogenic differentiation. Despite the limitations in some clones in the adoption of cell fates, most remained positive for common MSC markers, including CD29, CD44, CD73, CD90, CD105, and CD166 (Mareddy et al. 2007). Similar findings of population heterogeneity have been reported for stem cells derived from adipose tissue (Guilak et al. 2006), suggesting that this same phenomenon is present in progenitor cells of different sources. It has further been noted that the osteogenic lineage is predominant in clonal expansion studies; while a large percentage of clonal cell populations are capable of tridirectional and bidirectional differentiation, almost all cell colonies are capable of osteogenic differentiation (Halleux et al. 2001; Gronthos et al. 2003). Out of 100 immortalized clonal populations, Okamoto et al found no bidirectional adipo-chondrogenic clones (Okamoto et al. 2002). A recent study by Hardingham and colleagues specifically analyzed cartilage tissue formation from individual clonal populations of MSCs, and showed markedly different matrix forming capacity and retention of phenotype in individual clones (Murdoch et al. 2008). Additionally, work by Lennon et al demonstrated reduced differentiation toward osteogenic and chondrogenic lineages when the starting MSC population was diluted with fibroblasts past a certain threshold ratio (Lennon et al. 2000). Therefore, it is clear that isolated mesenchymal stem cell populations are heterogenic in their differentiation potential and that current cell surface markers are insufficient to describe the variable potential in these populations.

The above findings bring to bear the question of ‘real’ seeding density of MSCs in tissue engineered environments. Indeed, the heterogeneous cohort of cells may considerably reduce

the 'apparent' seeding density, as fewer cells in this population are truly capable of chondrogenic differentiation, and in this way may contribute to the reduction in functional potential observed. This principle is illustrated in Figure 3, where MSCs from the same population, within the same area of hydrogel, show dramatically different proteoglycan deposition in their pericellular space during early times of culture. Given this appreciation of heterogeneity, increasing the initial MSC seeding density of constructs may be one route towards reducing the functional discrepancy between MSCs and chondrocytes. Further, studies of pellet cultures suggest that cell-cell contact and/or communication play important roles in the initiation of chondrogenesis (Tuli et al. 2003). Several studies have taken this approach and examined the effect of varying seeding density on chondrogenesis (Ponticiello et al. 2000; Kavalkovich et al. 2002; Huang et al. 2004; Park et al. 2007; Hui et al. 2008; Huang et al. 2009). Results from these studies show that at low seeding densities (1–5 million cells/mL), increasing MSC seeding density increases the rate of matrix deposition on a per cell basis (Hui et al. 2008); in contrast, bulk biochemical content is insensitive to MSC seeding density at higher seeding densities (20#x02013;60 million cells/mL) (Mauck et al. 2006; Huang et al. 2009). We and others have demonstrated that at this high range of seeding densities, matrix deposition becomes markedly less efficient on a per cell basis (Kavalkovich et al. 2002) and mechanical properties do not improve with higher MSC seeding densities (Huang et al. 2009). These findings suggest that limitations in functional properties are not simply due to too few cells producing cartilage ECM at a high level, further underscoring the differences between chondrogenically differentiated MSCs and fully differentiated chondrocytes. Negative feedback mechanisms and nutrient limitations in these conditions may also act to limit MSC matrix deposition past a certain threshold. Work with chondrocyte-based constructs have shown that matrix synthesis rates decline after establishment of ECM (Buschmann et al. 1992), though it is unknown how this mechanism influences MSC chondrogenesis.

Expanding the Definition of MSC Chondrogenesis

Ultimately, the current limitations in MSC chondrogenesis may reflect our inability to instill the correct phenotype in these cells. At the molecular level, standard benchmarks of chondrogenesis include the expression of *sox 9*, aggrecan and type II collagen genes while at the tissue level, cartilaginous ECM composition is typically defined by the presence of proteoglycans and type II collagen, and the absence of type I collagen. While our understanding of MSC differentiation has progressed tremendously in the last several years and new markers of chondrogenesis have been elucidated (Boeuf et al. 2007), few studies have examined the molecular limitations of MSC chondrogenesis, particularly in terms of phenotypic stability and functional capability.

One way to think through this problem is to appreciate that the standard benchmarks of chondrogenesis as currently applied is just as reflective of the phenotype of 'transient' chondrocytes (which reside in the growth plate of developing joints and undergo hypertrophy and eventual ossification) as it is of the phenotype of 'permanent' chondrocytes (which reside in the articular cartilage and maintain a fixed chondrocytic phenotype through a lifetime of use). Indeed, recent studies suggest that under standard methods of induction, MSC chondrogenesis may better approximate the mutable and undesired phenotype of 'transient' or osteoarthritic chondrocytes rather than 'permanent' chondrocytes (Winter et al. 2003). For example, Pelttari et al showed that subcutaneous implantation of chondrogenically differentiated MSC pellets result in mineralized deposits within the original cartilaginous matrix, though it is unknown whether mineralization is due to the implanted MSC population or infiltration by host cells (Pelttari et al. 2006). Further evidence of this phenomenon is provided by Jukes et al, who pre-differentiated embryonic stem cells on ceramic particles toward the chondrogenic lineage and showed conversion toward an osteogenic phenotype after additional culture *in vivo* (Jukes et al. 2008). Similar results are also observed for MSCs on

these ceramic particles. Notably, in both studies, chondrocyte control groups maintain phenotypic stability under similar *in vivo* conditions and form additional cartilage matrix with no evidence of mineralization. These data further delineate the differences between differentiated MSCs and native articular chondrocytes.

In most MSC chondrogenesis studies, differentiation is induced under serum-free media conditions in the presence of TGF- β 3, a known inhibitor of matrix mineralization. The absence of exogenous TGF- β post-implantation may contribute to the observed phenotypic instability of MSCs; however, several *in vitro* studies suggest that the mere removal of this morphogen is not sufficient to induce hypertrophy or mineralization. Collectively, the data show that differentiated MSCs are able to maintain their differentiated state on a molecular level after a transient period of exposure to TGF- β (Caterson et al. 2001; Mehlhorn et al. 2006). Indeed, our recent data shows that mechanical and biochemical properties of formed constructs is maintained (or improved) on a seeding density dependent basis (Huang et al. 2009). Transdifferentiation of MSCs towards the osteogenic phenotype was successful *in vitro* only with the addition of thyroid hormone and β -glycerophosphate and reduction of dexamethasone, after TGF- β was removed (Mueller et al. 2008). Whether these factors are present in the *in vivo* environment, and at what levels, is currently unknown, though these studies point to the possibility of phenotypic conversion upon implantation.

Other *in vitro* studies pursue an alternative strategy, whereby chondrogenic induction is followed by a period of de-differentiation, prior to the application of osteogenic cues (Song et al. 2004; Song et al. 2006). From these studies, a clearer understanding of the genes modulated by chondrogenesis is emerging, although significant work remains. To identify the molecular events underlying the functional capabilities of MSCs, we recently conducted a genome-wide screen of chondrogenesis using microarray analysis. Direct comparisons of donor-matched chondrocytes and MSCs reveals a large number of genes that remain differentially regulated, even after chondrogenesis is fully developed (Huang et al. 2008), Figure 4. Data from this study show that standard cartilaginous markers are comparably expressed between the two cell types, further demonstrating that our current benchmarks for describing the chondrogenic phenotype is incomplete. Appreciation of these differences in phenotypic state, and development of enabling methods to overcome these gaps, will be essential in the functional formation of cartilage using MSCs.

Mechanical Stimulation of MSC-based Engineered Cartilage Constructs

To improve the functional outcome of MSC-based constructs and better instill the phenotypic traits associated with articular cartilage, one potential strategy for optimization is direct mechanical stimulation. Mechanical stimulation methods have been widely employed in chondrocyte-based cartilage tissue engineering, with many studies showing improvements in mechanical properties and biochemical composition when the loading conditions are appropriately tuned (Mauck et al. 2000; Connelly et al. 2004; Kisiday et al. 2004; Grad et al. 2005; De Croos et al. 2006; Hu et al. 2006; Appelman et al. 2009). The rationale for taking a similar approach for MSC-based constructs is easily identified from the crucial role mechanical forces play during normal joint development. For example, inhibition of muscle forces results in incomplete formation of the joint and mechanical signals modulate the expression of important molecular factors, such as PTHrP, which may regulate the phenotypic state of mesenchymal cells during development (Vortkamp et al. 1996; Mikic et al. 2000; Mikic et al. 2004; Chen et al. 2008). In addition, mechanical regulation remains important after birth, as cartilage undergoes additional remodeling and mechanical maturation with load-bearing use (Williamson et al. 2003; Williamson et al. 2003) and the maintenance of healthy cartilage and the retention of the chondrogenic phenotype in normal articular chondrocytes is dependent on mechanical loading (Chen et al. 2008). Further, compressive loading of chick limb-bud

mesenchyme cells (a distinct but related cell type to MSCs) in agarose enhances chondrogenesis; this response is modulated by both the frequency and duration of the applied load (Elder et al. 2000; Elder et al. 2001).

Taken together, these findings form the basis for mechanical preconditioning of MSC-based constructs for cartilage tissue engineering applications, and indeed, results from several studies indicate that MSCs are responsive to mechanical loading. For example, hydrostatic pressurization, an indirect loading modality, enhances the expression of chondrogenic genes in MSC pellets and MSC-seeded constructs (Angele et al. 2003; Miyanishi et al. 2006; Finger et al. 2007; Wagner et al. 2008). More direct modes of mechanical perturbation, both in compression and tension, have also been examined, however, the effects of these loading modalities on MSC chondrogenesis and the development of functional properties remains unclear. Early studies of MSC mechanotransduction have focused on short-term application of load and limited outcome measures to gene expression and matrix synthesis. In one of the first studies examining the relationship between mechanical stimulation and MSC differentiation, Huang and co-workers applied dynamic compression to MSC-seeded agarose and induced several transcription factors known to mediate TGF- β signaling, including sox 9, AP-1 and c-Jun (Huang et al. 2005). When MSC-seeded porous hyaluronan-gelatin constructs were subjected to compressive loading, the expression levels of chondrogenic genes were upregulated relative to non-loaded controls (Angele et al. 2004). In another study, short-term loading of MSC-laden agarose cultures in the absence of TGF- β increased aggrecan promoter activity, but decreased collagen type II promoter activity (Mauck et al. 2007). Consistent with this, Kisiday and colleagues recently demonstrated that 12 hours of continuous loading in the absence of TGF- β improved proteoglycan synthesis levels, though these values did not reach those attained with inclusion of TGF- β under free swelling conditions. In cultures loaded in the presence of TGF- β , matrix synthesis levels diminished (Kisiday et al. 2009). In these studies, application of load was initiated before elaboration of ECM, yet pre-culturing constructs before loading may also modulate cell properties. A single application of cyclic compression improved both collagen type II and aggrecan expression when MSC-seeded agarose gels were pre-cultured in TGF- β 1 containing media for 16 days; these effects were not observed with a pre-culture time of 8 days (Mouw et al. 2007). Under cyclic tension (10% strain, 1Hz), the rate of GAG synthesis was enhanced in MSCs seeded on collagen-GAG scaffolds (McMahon et al. 2008; McMahon et al. 2008), suggesting a positive role for cyclic tensile loading in chondrogenesis. However, tensile loading can also induced apoptosis of MSC-seeded silicon membranes at strains of 7.5% or greater (Kearney et al. 2008).

Collectively, these studies affirm that MSCs are mechanically sensitive and that MSC chondrogenesis can be modulated by mechanical stimulation. While these findings suggest that dynamic loading may be beneficial in inducing/enhancing chondrogenesis, and that the presence or absence of TGF- β may define this response, few studies have examined the effects of long-term loading or have assessed functional outcomes. One recent study showed that long-term mechanical compression impairs functional growth of MSC-based engineered constructs. When loaded daily for 42 days in TGF- β containing media, the compressive modulus and GAG content of MSC-seeded agarose constructs were considerably reduced compared to free-swelling controls (Thorpe et al. 2008). From this study, it appears that MSC and chondrocyte response to mechanical stimulation are not identical. Consistent with the above observations, we have observed that compressive loading of MSC-seeded agarose in the presence of TGF- β 3 improves aggrecan expression, though compressive mechanical properties and biochemical contents are significantly reduced over 21 days of continuous loading initiated when cultures are first established (Figure 5). Interestingly, GAG accrued on a per cell basis in these constructs was comparable between loaded and non-loaded samples, suggesting that loading may slow cell proliferation in early cultures without necessarily impairing MSC differentiation status.

Although these studies indicate that long-term compressive loading may be detrimental to the development of functional properties in MSC-based constructs, a recent study by Teracciano and co-workers provides evidence that pre-differentiation of stem cells may modulate the effects of compressive loading. In this study, embryonic stem cells embedded in a hydrogel environment responded adversely to applied load in the absence of TGF- β ; however, after a period of chondrogenic induction, applied mechanical load elicited positive effects on cartilaginous gene expression (Terraciano et al. 2007). To assess whether pre-differentiation may also modulate MSC response to compressive loading, we pre-cultured MSCs in agarose in the presence of TGF- β 3 over a period of three weeks prior to the application of load. Consistent with previous findings from our lab, MSC constructs improved mechanical properties and biochemical content over three weeks of free-swelling culture, reaching a compressive modulus of ~75 kPa (Figure 5). After three weeks of repeated compressive loading (5 days/week, 4 hours daily) in TGF- β 3 supplemented media, the compressive modulus of loaded constructs was ~150 kPa, compared to ~100 kPa of non-loaded samples. Notably, expression of cartilaginous genes peaked two weeks after the initiation of load (1.5 to 2-fold over control levels) before dropping below control levels, suggesting there may be a limitation to the mechano-responsivity of MSCs to repeated loading, or that assessment of molecular levels changes do not reflect the trajectory of construct maturation in terms of mechanical properties. In contrast to these findings with MSCs, loading of chondrocyte-seeded agarose after a period of pre-culture is only beneficial in the absence of TGF- β 3, highlighting yet another point of dissimilarity between these cell types (Lima et al. 2007). Taken together, these findings strongly suggest that the establishment of a chondrogenic phenotype and surrounding pericellular matrix prior to the initiation of loading is a crucial determinant of MSC response. A more complete understanding of the mechanotransduction pathways occurring during loading will enable us to better tailor our loading regime to optimize functional outcomes.

Conclusions and Future Directions

The last several years have advanced our understanding of MSC chondrogenesis and our ability to form, with these cells, engineered tissues possessing some degree of functional integrity. However, to date, we have yet to instill MSC-based constructs with mechanical properties matching that of native cartilage, or even that produced by chondrocyte-based constructs. Of further concern, the phenotype of induced MSCs may not be fixed in the chondrogenic lineage, with growing evidence suggesting that the commitment to phenotype is malleable and/or progressive, and dependent on the external environment. Successful implementation of MSCs for cartilage repair then will rest on our ability to resolve these gaps in our understanding of the molecular conversion of these cells and to promote and maintain native mechanical properties and phenotype. To accomplish this goal, several key enabling steps can be pursued by the field as a whole.

One clear area for improvement is the development of a better understanding of the heterogeneity of MSC populations and the profile of MSC chondrogenesis. The issue of homogeneity can be likened to a rowboat with only one oar; if a significant portion of the population fails to establish matrix, but consumes necessary nutrients, then functional matrix deposition will perforce be limited. Better surface selection methods, or the establishment of mechanical criteria within the stem cell populations, may significantly improve outcomes by populating forming constructs with MSCs that perform efficiently and in concert. In terms of profiling the molecular events associated with chondrogenesis, our consideration should not be limited towards optimizing the events that do take place, but also focus on the molecular events that fail to take place. Untold genes, with potentially critical functions in matrix maturation may fail to initiate or turn off in the appropriate sequence, and so, significantly impede tissue development. Work towards this end is certainly ongoing (Barry et al. 2001; Sekiya et al. 2002; Song et al. 2006; Boeuf et al. 2007; Yamane et al. 2007), and ensuring

proper comparisons to fully differentiated chondrocytes will shed new light into these processes. These differences in genomic or proteomic signatures will be instrumental in defining real benchmarks for evaluating chondrogenesis and our ability to instill the desired phenotype in these cells. An important point here is to make sure that the field is using the same definitions of success; a recent microarray analysis by Reddi and co-workers comparing growth plate with articular cartilage shows profound differences between these transient and permanent cartilages, respectively (Yamane et al. 2007). Since the permanent hyaline cartilage (and the chondrocytes within) shows a remarkable ability to first establish functional matrix and then resist progressive phenotypic conversion towards the osteogenic lineage, these cells in particular should serve as our 'gold standard' for chondrogenesis. Once established, these benchmarks can be utilized directly, for example via molecular interventions (Palmer et al. 2005), or used to identify optimal media formulations and loading regimes necessary to manufacture constructs with the appropriate functional properties, biochemical make-up and phenotypic traits of articular cartilage.

Another point worth noting, but not addressed in this review, is the fact that the composition of the mostly widely used media formulation for inducing chondrogenesis has been relatively fixed since the original description in the 1990s. While this media has been successful in inducing some aspects of chondrogenesis, limitations in this formulation may underlie the inability of MSCs to fully assume the chondrogenic phenotype. Several studies have examined the chondrogenic effects of various combinations of factors and supplements; however, these studies have been limited in scope (Awad et al. 2003; Indrawattana et al. 2004; Toh et al. 2005; Estes et al. 2006; Im et al. 2006; Longobardi et al. 2006). As a result, the capacity and potency of alternative factors to modulate chondrogenesis remains largely unexplored. To overcome these limitations, we have recently developed and validated a high-throughput screening method to identify small molecule modulators of chondrogenesis (Huang et al. 2008). Using this method, large numbers of chemical compounds, as well as their combinations, can be rapidly assayed to identify new factors that can induce or inhibit differentiation. These biochemical modulators, and the timing of their administration, may be useful in promoting the chondrogenic phenotype. Resources such as the Small Molecule Repository (SMR, a NIH-established collection of >200,000 molecules of unknown function) might prove a rich source of molecules that further MSC chondrogenesis.

In addition to these molecular and biochemical methods, the mechanical regulation of differentiation and matrix formation by MSCs is only starting to be considered. While early results showed much promise in terms of improving expression of certain key cartilage markers, conversion towards functional efficacy has been a slow process. Nevertheless, our recent work and that of others demonstrates that mechanical stimulation can improve maturation of engineered constructs based on MSCs. Ongoing work will need to address the most appropriate types, magnitudes, durations, and pre-culture periods that best promote the conversion of these mechanical signals towards productive matrix elaboration. No small measure of this will involve a much more detailed understanding of the signaling pathways that underlie these mechanotransduction events. Data so far suggests that these loading protocols must be tuned to the developmental 'state' of the forming tissues, and is highly dependent on the biochemical and biomaterial milieu in which the loading is applied.

Taken together, the promise of MSCs for cartilage tissue engineering far outweighs these limitations. While the remaining hurdles are significant, setting realistic expectations for this cell type, establishing sound comparisons and valid benchmarks, and employing rigorous analytical techniques will further these efforts, and ultimately yield a clinically relevant replacement cartilage for the repair of damaged or diseased joints.

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Figure 1.

Type II collagen deposition in chondrocyte (CH) - and MSC-seeded agarose constructs seeded at 10 million cells/mL after 56 days of *in vitro* culture in a chemically defined medium in the presence (CM+) or absence (CM-) of TGF- β 3. Scale Bar: 100 μ m. Data adapted with permission from Elsevier.

Figure 2.

(A) Equilibrium compressive modulus of MSC-seeded agarose, photo-crosslinked hyaluronic acid (HA), and self-assembling Puramatrix hydrogels (20 million cells/mL) after long-term culture in a chemically defined media containing 10 ng/mL TGF- β 3. Data represent the mean and standard deviation of 3-4 samples per time point; * indicates $p < 0.05$ versus Day 0. (B-D) Alcian blue staining demonstrates robust proteoglycan deposition in agarose (B), HA (C) and Puramatrix (D) hydrogels on day 56. Scale Bar: 100 μ m. Data adapted with permission from Mary Ann Liebert, Inc.

Figure 3.

Alcian blue staining demonstrates inhomogeneous pericellular accumulation of proteoglycans by MSCs seeded in photo-crosslinked hyaluronic acid (5% w/v). Constructs were cultured for 7 days in chemically defined media supplemented with 10 ng/mL TGF- β 3. Scale Bar: 100 μ m.

Figure 4.

(A) Principle component analysis and (B) heat map generated from microarray data showing differential gene expression (green = greater, red = lower) between undifferentiated MSCs in agarose on day 0 (M0), chondrogenically differentiated MSCs in agarose on day 28 (M28) and chondrocytes in agarose on day 28 (C28).

Figure 5.

Equilibrium compressive modulus of MSC-seeded agarose (20 million cells/mL) subjected to repeated dynamic compressive loading in the presence of 10 ng/mL TGF- β 3. Cyclic loading was initiated after 3 days or 3 weeks of pre-culture. * indicates significant difference from day 0, ** indicates significant difference from day 0 and free-swelling controls within each timepoint ($p < 0.015$).

Table 1

Examples of Engineered Cartilage Derived from MSCs and other Progenitor Cell Sources

BIOMATERIAL	CELL SOURCES	OUTCOME ASSAYS	MECHANICAL PROPERTIES	REFERENCE
Alginate	Human, bone marrow derived	Gene expression, histology, SEM, GAG content	NA	(Wang et al. 2009)
	Human	Gene expression	NA	(Xu et al. 2008)
Alginate (RGD-modified)	Bovine, bone marrow derived	Gene expression, biochemical analysis	NA	(Connelly et al. 2007)
Agarose	Bovine, bone marrow derived	Biochemical analysis, histology, compressive properties	Eq. modulus: 48 kPa Dyn. Modulus: 800 kPa	(Mauck et al. 2006)
	Bovine, bone marrow derived	Biochemical analysis, histology, tensile properties	Tensile modulus: 363 kPa Toughness: 420 kPa Ultimate Strain: 63%	(Huang et al. 2008)
Chitosan scaffold	Human, bone marrow and adipose derived	Gene expression, histology, SEM	NA	(Seda Tigli et al. 2009)
Collagen type I	Human, bone marrow derived	Gene expression, biochemical analysis, histology	NA	(Dickhut et al. 2008)
Collagen type II	Human, bone marrow derived	Histology	NA	(Chang et al. 2007)
Fibrin	Human, bone marrow derived	Gene expression, biochemical analysis, histology	NA	(Dickhut et al. 2008)
	Human, bone marrow derived	Gene expression, proliferation	NA	(Pelaez et al. 2008)
Gelatin	Human, bone marrow derived	Histology, biochemical analysis	NA	(Ponticello et al. 2000)
	Human, adipose derived	Biochemical analysis, histology, compressive and shear properties	Eq. compressive modulus: ~15 kPa Eq. shear modulus: ~4 kPa Complex shear modulus: ~6 kPa	(Awad et al. 2004)
Hyaluronic Acid	Bovine, bone marrow derived	Biochemical content, compressive properties	Eq. Modulus: 60 kPa Dyn. Modulus: 40 kPa	(Erickson et al. 2009)
	Human, bone marrow derived	Gene expression, biochemical analysis, histology, compressive properties	Eq. modulus: 50–60 kPa	(Chung et al. 2009)
	Human, bone marrow derived	Gene expression, biochemical analysis, histology, immunohistochemistry	NA	(Nesti et al. 2008)
Matrigel	Human, bone marrow derived	Gene expression, biochemical analysis, histology	NA	(Dickhut et al. 2008)
oligo(poly(ethylene glycol) fumarate) (OPF) scaffolds	Rabbit, bone marrow derived	Gene expression, confocal microscopy	NA	(Park et al. 2009)
poly(ϵ -caprolactone) (PCL) nanofiber scaffolds	Human, bone marrow derived	Gene expression, biochemical analysis, histology, SEM	NA	(Li et al. 2005)
poly(ethylene glycol) (PEG)	Human, bone marrow derived	Gene expression, immunohistochemistry	NA	(Chung et al. 2009)

BIOMATERIAL	CELL SOURCES	OUTCOME ASSAYS	MECHANICAL PROPERTIES	REFERENCE
	Human, bone marrow derived	Biochemical analysis, histology, immunohistochemistry	NA	(Buxton et al. 2007)
Poly(ethylene oxide) diacrylate (PEODA) with collagen mimetic peptides	Goat, bone marrow derived	Gene expression, biochemical analysis, histology	NA	(Lee et al. 2008)
Polyglycolic acid (PGA)	Rabbit, bone marrow derived	Histology, immunohistochemistry	NA	(Zhou et al. 2008)
poly lactic-co-glycolic acid (PLGA) scaffold	Rabbit, bone marrow derived	Histology, biochemical analysis	NA	(Zhang et al. 2009)
poly(l-lactic acid) (PLLA) nanofiber scaffolds	Human, bone marrow derived	Gene expression, histology, compressive properties	Eq. Modulus: 12 kPa	(Janjanin et al. 2008)
Puramatrix	Bovine, bone marrow derived	Biochemical analysis, compressive properties	Eq. Modulus: 80 kPa Dyn. Modulus: 120 kPa	(Erickson et al. 2009)
Silk fibroin scaffold	Human, bone marrow derived	Gene expression, biochemical analysis, histology, compressive properties	Aggregate modulus: 40 kPa	(Hofmann et al. 2006)
	Human, bone marrow and adipose derived	Biochemical analysis, histology	NA	(Seda Tigli et al. 2009)