Cell sources for articular cartilage repair strategies: shifting from mono-cultures to co-cultures

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Conflicting interest: No competing interest exists.

KEYWORDS: Review; articular cartilage, repair, cell sources, chondrocytes, MSCs, hypertrophy, dedifferentiation, co-culture, cell therapy, tissue engineering.

Abstract.

The repair of articular cartilage is challenging due to the sparse native cell population combined with the avascular and aneural nature of the tissue. In recent years cartilage tissue engineering has shown great promise. As with all tissue engineering strategies, the possible therapeutic outcome is intimately linked with the used combination of cells, growth factors and biomaterials. However, the optimal combination has remained a controversial topic and no consensus has been reached. In consequence, much effort has been dedicated to further design, investigate and optimize cartilage repair strategies. Specifically, various research groups have performed intensive investigations attempting to identify the single most optimal cell source for articular cartilage repair strategies. However, recent findings indicate that not the heavily investigated mono cell source, but the less studied combinations of cell sources in co-culture might be more attractive for cartilage repair strategies.

This review will give a comprehensive overview on the cell sources that have been investigated for articular cartilage repair strategies. In particular, the advantages and disadvantages of investigated cell sources are comprehensively discussed with emphasis on the potential of co-cultures in which benefits are combined while the disadvantages of single cell sources for cartilage repair are mitigated.

Introduction

Mature articular cartilage is composed of an abundant extracellular matrix that is sparsely populated by chondrocytes. Articular cartilage is avascular and aneural and the native chondrocytes are largely in cell cycle arrest. As a consequence, it is challenging for this tissue to suitably respond to traumatic injury. Cartilage damage inevitably results in altered biomechanics of the joint and joint instability, which shift joint homeostasis towards catabolism (1, 2). If left untreated, this may ultimately lead to joint failure. As a result, the recalcitrant capacity of articular cartilage to self-heal acquired injuries drives research focused on (novel) cartilage reparative and regenerative treatments.

Current treatments of traumatic cartilage defects include osteochondral grafting, bone marrow stimulation techniques and more recently cell based therapies (3-6). Osteochondral autografting is particularly suitable for smaller lesions, but is associated with donor-site morbidity (7). Osteochondral allografting can be considered for larger defects, but is associated with graft failure and immune reactions (8, 9). Bone marrow stimulation techniques such as micro-fracturing and micro-drilling are used for defects smaller than 2-4 cm^2 with little or no bone loss and are associated with the formation of fibrocartilage (10). The most common form of cell-based therapies is autologous chondrocyte implantation (ACI). This treatment is based on the implantation of expanded autologous chondrocytes, which were isolated from a biopsy that was obtained from a non-load bearing site of the same donor. Since ACI is dependent on the violation of intact cartilage as well as ex-vivo chondrocyte expansion, it is associated with donor-site morbidity and loss of chondrogenicity of cultured chondrocytes known as dedifferentiation (4).

Consequently, a myriad of alternative cell sources other than culture expanded articular chondrocytes have been investigated to circumvent these side-effects and thereby to improve the therapeutic outcome of cartilage repair strategies. In addition to several types of chondrocytes derived from a number of hyaline cartilage subtypes, the use of multi-potent cells with chondrogenic potential derived from various tissue sources has been proposed. In addition to conventional mono-culture the approach of co-cultures has gained significant attention. Interestingly, co-cultures have been shown to be able to outperform their respective single cultures. Unfortunately, in depth understanding and clinical translation of this phenomenon has remained wanted.

This review will discuss the different cell sources dominating the field of cartilage tissue engineering and highlights recent advances of co-culture approaches.

Autologous articular chondrocytes: cell number versus dedifferentiation

Conventional cell-based cartilage repair therapies characteristically use autologous articular chondrocytes. These cells are harvested from biopsies of macroscopically intact cartilage derived from a non-weight bearing part of the joint (11). Removal of a biopsy from healthy articular cartilage can potentially lead to secondary osteoarthritis. However, more thorough studies with a longer follow-up are required to understand the full extent of the consequences of harvesting biopsies (12). To minimize the chance of developing secondary osteoarthritis the biopsy size is limited. In consequence, the biopsy yields insufficient amounts of chondrocytes to allow for direct filling of the defect. Therefore, chondrocytes are expanded *in vitro* before reimplantation. Unfortunately, expansion is associated with progressive loss of the chondrogenic phenotype and results in dedifferentiated fibroblast-like chondrocytes (13). We recently

demonstrated that articular chondrocyte dedifferentiation is a continuous, progressive and multi-signaling-pathway-based process. Moreover, the potential to redifferentiate is also gradually lost and might be in part caused by epigenetic mechanisms such as DNA methylation. The loss of the chondrocyte phenotype during monolayer expansion is suggested to be dependent on the matrix elasticity of the culture environment (14). As this process can impede the therapeutic outcome, the prevention of dedifferentiation and/or the induction of redifferentiation have been intensively investigated. Investigated strategies include exposure to optimized environmental properties like reduced oxygen levels (15) and physiological tonicity of the culture medium (16), dynamic non-adherent culturing as aggregates (17), decreased seeding density (18), three dimensional expansion of cells using e.g. microcarriers (19), varying the elasticity of the growth surface to simulate cartilage stiffness (14) or pore-size of carrier material (20), culture on predeposited extracellular matrices of e.g. synovium-derived stem cells (21), the use of medium preconditioned by primary chondrocytes or MSCs (22-24) or supplementation of the expansion medium with exogenous recombinant growth factors, like FGF-2 (25). In general, these strategies led to improved expression of chondrogenic markers and a (partial) reduction of dedifferentiation markers. However, as large, comprehensive and well controlled comparative studies are largely lacking, consensus on an optimal strategy remains wanted.

Dedifferentiation can also be limited or even prevented by reducing or omitting the expansion phase. This idea is based on implanting fewer chondrocytes that are less expanded and have therefore a superior chondrogenic performance (26-28). Although this approach demonstrated to be able to regenerate cartilage, it results in mechanically

weaker cartilage when compared to implantation of higher densities of chondrocytes expanded over prolonged culture times in the same defect size. By reducing or omitting the amount of dedicated cell culture time, it might be possible to improve the costs-effectiveness of chondrocyte-based cell therapy and reduce the time between taking the biopsy and implanting expanded cells in a second surgical procedure.

Non-autologous articular chondrocytes: morbidity versus immune response

Allogenic, or even xenogenic, articular chondrocytes can be considered as an alternative cell source of which the isolation does not inflict additional damage to the joint. Advantageously, a larger number of chondrocytes can be obtained by taking larger biopsies and/or pooling different, potentially younger, donors. This might limit or obsolete the expansion phase, leading to a better preservation of the chondrocyte phenotype, and could potentially reduce inter-patient variation of the therapeutic outcome. Naturally, serious consideration should be given to drawbacks such as disease transmission and immune rejection (29). Indeed, chondrocytes constitutively express histocompatibility complex class I at their cell surface and demonstrate cytokine inducible expression of histocompatibility complex class II (30). Moreover, it has been shown that chondrocytes can interact with immune cells (31). Several reports have described serious immune responses after allogenic chondrocyte implantation in full thickness defects where the access to the bone marrow increases the risk for immunological reactions (32-34). Consequently, the clinical feasibility of allogenic chondrocytes implantation is questionable. In contrast, osteochondral allograft demonstrated only minor immunogenic signs in human clinical trials (35, 36). On the one

hand the immunological responses might be caused by the osseous part of the graft with residing bone marrow (9), whereas on the other hand the protective environment of the native cartilage will limit immunological reactions. The avascular and highly dense nature of the extra cellular matrix might be able to limit or prevent the invasion of immune cells from the joint to interact with chondrocytes. Indeed, isolated chondrocytes appear to be protected from an immune response when encapsulated in biocompatible biomaterials or when allowed to form a new extracellular matrix *in vitro* (37, 38). Due to their more wide availability and the limited immunogenic risk when embedded in an extracellular matrix, non-autologous articular chondrocytes can be still considered as a potentially interesting cell source for matrix assisted chondrocyte transplantations.

Non-articular chondrocytes: morbidity versus phenotype

Next to articular chondrocytes, several alternative chondrocyte sources have been investigated to design cartilage repair strategies that do not impose additional damage to the articular cartilage. In adults, other sources of (non-articular) hyaline cartilage are located in the nose, ribs, larynx, trachea and bronchi. In particular, costal and nasoseptal chondrocytes have been extensively investigated (29, 39-41). Both costal and nasoseptal cartilage biopsies, respectively harvested from the ribcage or from the septum of the nose, grant a higher cell yield of chondrocytes. The proliferation rate of these chondrocytes is increased compared to articular chondrocytes (42). Although they both undergo dedifferentiation during expansion they appear more susceptible to redifferentiation (43, 44). Moreover, transplantation of costal chondrocytes in full thickness defects results in the production of neocartilage (45). Unfortunately, both costal and nasoseptal

chondrocytes are known for their ability to ossify when reimplanted (40, 46, 47). The different anatomical location and function of costal and nasoseptal cartilage might underlie their limited potential to form articular cartilage. In particular, the mechanical loading in the articular cartilage is frequent and highly compressive, whereas costal and nasoseptal cartilage experiences at best low tensile loads.

Auricular chondrocytes derived from elastocartilage of the ear are as well considered as a possibly interesting cell source for cartilage repair strategies. Native and freshly isolated auricular chondrocytes typically express elastin. Although, upon *in vitro* culture the expression of elastin is gradually lost (41, 48), it has been reported that the elastin expression can be regained when implanted *in vivo* (49). Whether the expression of elastin will negatively influence the mechanical properties of neocartilage in an articular cartilage defect remains to be clarified. Like nasoseptal and costal cartilage, isolated auricular cartilage provides a higher cell yield per gram of biopsied tissue and have a higher proliferation rate compared to articular cartilage. Auricular chondrocytes are prone to dedifferentiation and are susceptible to redifferentiation (42, 50). Mild forms of ossification have been observed after reimplantation (40). Even though auricular cartilage has a rather non-loaded, supportive function for the external ear, it has been found that auricular chondrocytes express lubricin, a mucinous glycoprotein essential for lubrication of the joint (41). Therefore, auricular chondrocytes are a cell source of considerable interest, in particular for repair or reconstruction of the superficial zone of articular cartilage.

Meniscal chondrocytes derived from the meniscal fibrocartilage also express lubricin and have been investigated in cartilage repair strategies (41, 51). However,

meniscal chondrocytes scarcely express collagen II, and have limited potential to secrete glycosaminoglycans. Consequently they generate neocartilage with inferior mechanical properties as compared to articular cartilage or other chondrocyte cell sources (52). Like ACI, the use of meniscal chondrocytes is based on cells isolated from a biopsy and may predispose to secondary OA, as it can induce joint instability (53). Additionally, the meniscus is partially exposed to the same catabolic environment as the articular cartilage, which deteriorates the chondrogenic behavior of the meniscal chondrocyte (54). As meniscal chondrocytes have similar disadvantage compared to articular chondrocytes and produce inferior neocartilage compared to these cells, they are an unlikely cell source for articular cartilage repair strategies.

Taking all available alternative chondrocyte populations into consideration, it is obvious that none of the alternative chondrocyte sources are exposed to a mechanical challenge, which is comparable to the stimuli present in the articular joint. Nevertheless, chondrocytes from alternative sources surpass articular chondrocytes in availability, cell yield and proliferation capacity and might be suitable for patient groups where the access to articular chondrocytes is limited.

Non-chondrocyte cell sources: morbidity versus control of differentiation

Recently much research has been dedicated to cell sources other than articular chondrocytes. This is mainly due to articular chondrocyte's low availability and its limited expansion capability without loss of function. These alternative cell sources include amongst others synovial fibroblasts, periosteocytes and multipotent progenitor cells.

Synovial fibroblasts are a part of the natural repair response to articular defects, as these cells tend to fill up non-treated defects with a fibrous matrix (55). Unfortunately, this matrix is, like the matrix produced by meniscal chondrocytes, mechanically weak and predominantly consists of collagen type 1 (56). Periosteocytes, depending on their site of isolation, possess chondrogenic potential that allows the formation of neocartilage (57). However, it has been suggested that this potential is inversely correlated with age. (58). In general, synovial fibroblast and meniscal chondrocytes have shown less promise compared to pluripotent/multipotent progenitor cells as an non-chondrocyte cell source.

Pluripotent cells such as embryonic stem cells and induced-pluripotent stem cells are able to form *de novo* articular-like cartilage and can in theory be considered for cartilage repair strategies (59, 60). However, gaining absolute control on the prevention of teratoma formation is paramount when using these cells (61, 62). Until such control is acquired, it is unlikely that these cell sources will be clinically approved for treatment of non-lethal diseases such as articular cartilage repair.

The best characterized progenitor cell sources for articular cartilage repair include mesenchymal stromal cells (MSCs) derived amongst others from bone marrow, periosteum, synovium, synovial fluid, adipose tissue, bucal fat pad, infrapatellar fat pad and osteoarthritic cartilage (63-67). Many other MSCs sources have remained largely uninvestigated for cartilage repair strategies and include amongst others umbilical cord blood**,** menstrual blood, muscles, ligaments, wartons jelly, amnion, chorion, breast milk and tonsil (68-75). Important factors deciding on which MSC source to use should not only be based on chondrogenic potential and phenotype, but also on cell yield,

accessibility, availability, age-related function decline, donor-site morbidity and acquisition costs.

Large inter-donor variation is a general complication encountered in all multipotent cell sources (76, 77). This is exacerbated by the influence of temporal culture conditions, methods of harvest and disputably donor age (57, 78-81). It has been suggested that distinct gene expression profiles might reflect their (chondrogenic) differentiation potential (82). However, the highly desired markers for chondrogenic differentiation potential of undifferentiated MSCs have so far remained undiscovered. Additionally, expansion of multipotent cells negatively affects chondrogenesis and might thereby possibly further confound therapeutic outcome (83, 84).

MSCs are a heterogeneous population of cells. In consequence, both the maintenance of multipotency and chondrogenic potential of MSCs are greatly influenced by selection criteria, culture conditions in the expansion phase and optionally the differentiation phase. Criteria for the selection of MSCs were defined by the Society for Cellular Therapy in 2006. These criteria require adherence to tissue culture plastic, the expression of a panel of cell surface markers ($CD90^+$, $CD105^+$, $CD73^+$, HLA-DR^-, $CD45^-$, $CD34^-$,$CD11b^-$, $CD19^-$) and the ability to differentiate into the adipogenic, osteogenic and chondrogenic lineage (85). Since then several further potential selection possibilities were published, including additional surface markers and distinct populations of MSCs that are found within the isolate (86-88). The classical view on optimizing the culture of mammalian cells is based on medium composition where much research has been focused on medium type and medium supplements (89-94). Non-autologous medium supplements might hamper the clinical applicability of tissue

engineering strategies. Presently much attention is paid towards optimizing physiological factors, which influence cell behavior and thus therapy outcome, such as mechanical stress, substrate stiffness, substrate coating or chemistry and incubator gas composition in particular oxygen levels (95-103). Other physiological variables such as tonicity have remained largely uninvestigated.

Rather than using MSCs for chondrogenic differentiation and direct production of neocartilage, one can also take advantage of the immunomodulatory or trophic properties of MSCs (104). It is suggested that after reimplantation differentiated MSCs continue to modulate the immune response (105). This might be of high importance in allogeneic treatments (106). However, whether this modulation proves to be sufficient in tissues that are scarce in cells and rich in matrix, such as articular cartilage, remains to be further studied. Nonetheless, using allogeneic strategies allow for a decrease in the therapeutic inter-donor variation via the use of pools of multiple donors or selection of well performing donors.

Intra-articular injection of MSCs in a degenerating joint improves joint function and retards the development of osteoarthritis compared to untreated controls (107). However, tracking experiments have shown that only a fraction of the injected MSCs are integrated in or located near the affected cartilage (108). In fact, most MSCs are located in the joint capsule or migrated from the joint to seemingly unrelated tissues such as thymus, tongue, stomach, duodenum, jejunum and colon (109). Together this suggests that the MSCs at least partially act via an indirect mechanism, most likely via the secretion of trophic factors. Indeed, *in vitro* co-culture experiments demonstrated the anabolic effects of MSCs-derived trophic factors on chondrocytes (24). However,

whether the same trophic factors are responsible for the anabolic effects observed *in vivo* remains to be proven.

Although MSCs can be differentiated in many different cell types and might contribute to tissue repair via released factors, it remains to be noted that their differentiation process cannot be fully controlled *in vivo*. Spontaneous sarcoma formation has been observed in long term expanded murine MSCs and after injection in mice (110, 111). Although this has not yet been reported in humans, the lack of full control over the behavioral phenotype of the MSCs remains a potential concern. For example, injections of MSCs in cancer therapy has both been described to restrict tumor growth by decreasing the division rate of benign cells as well as augment tumor growth by stimulating angiogenesis (112). These types of contradictions are not uncommon and underline the lack of deep understanding of the in-vivo behavior of MSCs. Absolute control of the selection and differentiation process of MSCs is essential if MSCs are to be used routinely for cell therapy such as articular cartilage repair. It can be conceived that more stringent selection criteria for the isolation of MSCs will result in a more homogenous and controllable cell source. However, this would also result a more prolonged expansion phase as many cells will be discarded, which might lead to (partial) loss of multipotency.

In vitro MSCs are differentiated into chondrocytes in serum free media supplemented with transferrin, insulin sodium selenite, Transforming Growth Factor beta 1 or 3 (TGF-β 1 or 3), dexamethasone (113) and in some studies with BMP6. Since the optimal combination and concentration of growth factors remains unclear, chondrogenic differentiation of multipotent cells such as MSCs typically results in the formation of

neocartilage that is dissimilar to mature articular cartilage. However, it bears a striking phenotypical resemblance to fetal cartilage (114, 115). Moreover, chondrogenic differentiation of MSCs results in a gene expression profile that better resembles growth plate chondrocytes, which can differentiate into hypertrophic cartilage, than articular chondrocytes (116). Indeed, cartilage formed by differentiated MSCs displays typical signs of hypertrophic differentiation (117-119). In line with this, standard differentiation protocols currently used for chondrogenic differentiation of MSCs *in vitro*, are able to induce the expression of chondrogenic genes, like aggrecan and collagen 2, but do not express the recently identified articular cartilage enriched genes, like DKK1, GREM1 and FRZB. These secreted antagonists were proven to function as inhibitors of hypertrophic differentiation (116). Consequently, chondrogenically differentiated multipotent and pluripotent cells undergo endochondral ossification upon subcutaneous implantation (60, 120). Although this phenomenon is of notable interest for bone repair strategies, it is highly undesirable for cartilage repair strategies. Moreover, it demonstrates that current differentiation protocols, at least *in vitro*, are insufficient to yield articular-like cartilage and require improvement for reproducible cartilage repair strategies. Finally, it is of importance to note that the formation of functional neocartilage has been witnessed in orthotopic repair in animal models. This suggests that the joint microenvironment contains instructive stimuli for the formation of permanent articular cartilage (121).

Co-cultures: combinatorial advantages versus current knowledge

Performance of cell sources in cartilage formation can be augmented by making use of co-culture strategies. Co-cultures of different cell sources are based on the idea that the multi-signal events *in vivo* cannot be perfectly mimicked by adding a limited variety of growth factors to a mono-culture of which the optimal cocktail remains largely elusive. This problem can be circumvented by the introduction of another cell source in the culture. In this way, cells are exposed to a wider variety of stimuli. Moreover, the stimuli are based on autologous non-recombinant (secreted soluble) factors. In consequence, they could be considered as a preferred way of stimulation, as it omitting the remaining issues regarding the use of non-autologous recombinant factors in clinical settings.

Three decades ago, the first co-culture experiments for cartilage tissue engineering were performed. First co-culture experiments mainly focused on revealing the pathological mechanisms of cartilage destruction by mixing chondrocytes with cell types that are potentially involved in cartilage catabolism (122, 123). More recently, co-cultures that include chondrocytes have been investigated for their capacity to enhance neocartilage formation. In general three (partially overlapping) categories can be identified: i) co-cultures with unilateral beneficial effect of one cell type on neocartilage formation; ii) co-cultures with mutually beneficial effects on neocartilage formation; iii) co-cultures based on cell types with unique features that do not (directly) affect the behavior of the other cell type (Figure 1).

Expansion of isolated autologous chondrocytes is commonly required to obtain sufficient amount of cells for reimplantation. This expansion induces dedifferentiation of the chondrocytes. Interestingly, co-culturing expanded dedifferentiated chondrocytes

with (previously frozen) primary chondrocytes can (partially) reverse their loss of phenotype (24, 124, 125) (Figure 1A). This redifferentiation appears to induce a stable phenotype; withdrawal of the exposure to primary chondrocytes does not lead to reversal of the effect (126). Alternatively, articular chondrocytes have been co-cultured with non-articular chondrocytes (127). However, so far little attention has been focused on these types of chondrocyte-chondrocyte co-cultures. In consequence, many questions still linger before an in-depth understanding of their actual therapeutic value can be obtained.

Presently, the most common co-cultures experiments investigate the effect of co-culture on the differentiation of the used cell types, as well as on improving the tissue formation in engineered constructs. In 1999 Jikko et al. showed that co-culturing growth plate chondrocytes with articular chondrocytes inhibited the terminal differentiation of growth plate chondrocytes (128). In line with this, it has been demonstrated that addition of articular chondrocytes to MSCs inhibits hypertrophic differentiation of the latter. Although the mechanism behind this phenomenon remains largely unknown, some evidence suggests that this effect is mediated via parathyroid hormone-related protein (129). However, alternative explanations such as articular cartilage derived factors inhibiting hypertrophic chondrocyte differentiation have remained uninvestigated (116).

The first studies in which MSCs were combined with other cell sources to provide a distinct effect included the vascularization of bone tissue engineered constructs (130, 131) and improvement of matrix deposition in degenerative discs (132). In contrast, in cartilage repair strategies MSCs were initially used to reduce the amount of chondrocytes needed or to omit their use all together. Fascinatingly, *chondro-induction* was observed in these experiments; superior neocartilage was formed by the combination of two different

cell-types as compared to either cell type alone (Figure 1B) (24, 133). In this context, the co-culture of articular chondrocytes and MSCs is most commonly studied. It is claimed that this phenomenon can be explained by the induction of chondrogenic differentiation of MSCs by articular chondrocytes (65, 134-136). Indeed, the addition of articular chondrocyte-conditioned medium is able to instigate chondrogenic differentiation of MSCs (137). However, recent findings demonstrate that chondrocyte proliferation is enhanced by the presence of MSCs (24, 136). The MSCs accomplish this effect by acting as a trophic mediator. Moreover, while chondrogenic differentiation of MSCs can be triggered by chondrocytes, it also induces apoptosis in the MSCs (24). Although the majority of MSCs will undergo apoptosis, the remaining fraction displays a strong chondrogenic phenotype (24, 136). This effect of MSCs is mediated by an as yet unidentified soluble secreted mediator. This results in a progressive disappearance of the MCSs from the original co-culture. Further investigation has to demonstrate if MSCs eventually completely vanish from the co-culture. If indeed this holds true, it can be considered as a beneficial factor for the use of (pools of) allogeneic MSCs. Moreover, if MSCs indeed predominantly stimulate neocartilage formation indirectly by acting as a source of trophic factors, one might consider MSC implantation sites within the joint other than the cartilage defect as the secreted factors diffuse to the cartilage via the synovial fluid. Regardless, in light of these novel findings the direct contribution of the MSCs to cartilage matrix deposition is still unclear. In addition, the question whether *chondro-induction* is induced due to exposure of factors normally produced by the opposite cell type or whether the co-culturing induces the expression of factors otherwise not expressed in either cell type remains to be answered.

Alternative strategies to improve neocartilage formation using co-cultures include the generation of articular cartilage's zonal architecture (Figure 1C). In recent years novel cartilage repair strategies have been designed that aim at mimicking this anisotropic organization (138, 139). Co-cultures in combination with multilayer three-dimensional printing or layer-by-layer methodology using hydrogels that are able to covalently link by residual reactive residues e.g. dextran-tyramine gels (140-143) can further enhance these strategies. Calcifying cells, or their precursors, can be used for the deep zone, intensive extracellular matrix producing calcification resistant cells for the middle zone, and less intensive extracellular matrix producing and lubricin secreting cells for the superficial zone. Furthermore, for example, osteoblasts and chondrocytes can be co-cultured in different regions of a construct to allow the formation of an osteochondral interface (144, 145). However, as only a scarce number of studies have been reported on such an approach, the feasibility remains to be determined.

Conclusion

The therapeutic outcome of cell-based therapies does not solely rely on the performance of the implanted cells. It also heavily relies on the expansion conditions and exogenous stimuli. In addition, functionalized biomaterials currently under development and which can be used as carriers for cell implantation, are expected to provide a platform for controlling cellular phenotype in the near future. Subsequently, these factors can result in dissimilar responses in different cell sources. In short, much research remains to be performed in order to identify the combinations leading to optimal clinical results. The wide range of cell sources available to cartilage tissue engineers grants an optimal starting point for future improvement of cartilage repair strategies. Specifically,

the combination of different cell sources currently holds great promise as it is able to grant combinatorial advantages by combining the benefits while mitigating the disadvantages of unique cell types.

Acknowledgements

The authors gratefully acknowledge the support of the TeRM Smart Mix Program of the Netherlands Ministry of Economic Affairs and the Netherlands Ministry of Education, Culture and Science.

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Figure 1: Different types of chondrocyte based co-cultures. (A) The addition of freshly isolated chondrocytes promotes the redifferentiation of expanded dedifferentiated chondrocytes. (B) Co-culturing MSCs with chondrocytes results in the enhanced proliferation and matrix deposition of the chondrocytes via MSC-derived soluble factors. Additionally, chondrocytes induce controlled cell death of MSC, which results in a strong decline of MSCs in the co-culture. The remaining MSCs undergo chondrogenic differentiation, which is driven by chondrocyte-derived soluble factors. (C) Layered

deposition of different cell types can be utilized to obtain a zonally structured construct, which display unique features in the distinct zones. For example, layering lubricin producing cells, chondrocytes and bone forming cells can be explored to form an osteochondral construct that mimics the native tissue.