JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE
J Tissue Eng Regen Med 2007; **1**: 170–178.
Published online 17 April 2007 in Wiley InterScience (www.interscience.wiley.com) **DOI:** 10.1002/term.19

REVIEW ARTICLE

Co-culture in cartilage tissue engineering

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Abstract

For biotechnological research *in vitro* in general and tissue engineering specifically, it is essential to mimic the natural conditions of the cellular environment as much as possible. In choosing a model system for *in vitro* experiments, the investigator always has to balance between being able to observe, measure or manipulate cell behaviour and copying the *in situ* environment of that cell. Most tissues in the body consist of more than one cell type. The organization of the cells in the tissue is essential for the tissue's normal development, homeostasis and repair reaction. In a co-culture system, two or more cell types brought together in the same culture environment very likely interact and communicate. Co-culture has proved to be a powerful *in vitro* tool in unravelling the importance of cellular interactions during normal physiology, homeostasis, repair and regeneration. The first co-culture studies focused mainly on the influence of cellular interactions on oocytes maturation to a pre-implantation blastocyst. Therefore, a brief overview of these studies is given here. Later on in the history of co-culture studies, it was applied to study cell–cell communication, after which, almost immediately as the field of tissue engineering was recognized, it was introduced in tissue engineering to study cellular interactions and their influence on tissue formation. This review discusses the introduction and applications of co-culture systems in cell biology research, with the emphasis on tissue engineering and its possible application for studying cartilage regeneration.

Received 7 December 2006; Revised 19 February 2007; Accepted 28 February 2007

Keywords co-culture tissue engineering; cell interactions; chondrocyte cartilage

1. History of co-culture studies

Over the last four decades, co-culture has been used extensively in biological research to investigate cellular interactions and cell function (Lawrence *et al*., 1978; Chan and Haschke, 1982; Murray and Fletcher, 1984). The very first co-culture study reported heterologous communication by means of gap junctions between combined rat ovarian granulosa cells and mouse myocardial cells (Lawrence *et al*., 1978). Thereafter, many co-culture studies focused on reproducing the natural embryonic development of pre-implantation embryos *in vitro* (Khurana and Wales, 1987; White *et al*., 1989). These experiments were performed not only to study pre-implantation development, but also to support fertilization programmes for clinical application (Menezo *et al*., 1990; Bongso *et al*., 1991). Research on the influence on normal development of co-culturing somatic cells with pre-implantation embryos resulted in defined co-culture protocols for clinical applications that are still used today (Sutton *et al*., 2003; Mercader *et al*., 2003). Until recently, the co-culture of fertilized oocytes with tubul ampullary cells, endometrial epithelial, oviduct endothelial or trophoblastic cells, or specific cell lines such as the Vero cell line, was considered to be the most viable way to support oocytes maturation towards implantable blastocysts (Bongso *et al*., 1991; Menezo *et al*., 1990; Barmat *et al*., 1999). Pluripotent embryonic stem cells in the embryo maintained their integrity during co-culture *in vitro*, while factors released from a supporting somatic cell type were necessary for their normal development *in vitro*. Studies on co-culture of preimplantation embryos with somatic cells identified signals exchanged by the 2 cell types, which support normal embryonic development.

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Further research led to successful reproduction of the influence of these signals on normal embryonic development with carefully composed media. (Urman and Balaban, 2005). If and how the physiology or differentiation of somatic cell types in co-culture are influenced in ways similar to those described for embryonic cells was discussed in subsequent publications.

The following sections review the nature of cellular interactions and their influence on cell physiology and/or differentiation. Being able to influence cellular interactions is of great interest in tissue engineering because, through these interactions, tissue formation of one or all cell types is regulated. The goal for tissue engineering is to simulate and stimulate natural physiology and differentiation of cells in order to engineer a tissue *in vitro* or induce the formation of a repair tissue *in situ*. The cellular interaction involved in tissue engineering most likely involves cell–cell communication. Other cellular interactions include cell–extracellular matrix or cell–biomaterial interactions, which play important regulating roles in normal cell physiology and differentiation. Cellular interactions examined in co-culture studies have mainly focused on cell–cell communication.

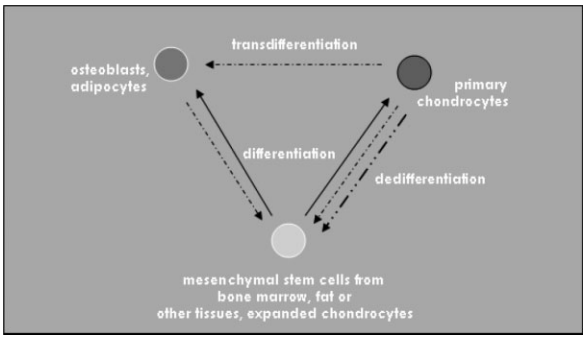


Figure 2. Differentiation, dedifferentiation and transdifferentiation of cells occurring *in situ* or *in vitro*. Differentiation (solid arrows) is the process by which cells undergo a change towards a more specialized form or function. Dedifferentiation (dashed arrow) is the regression of a specialized cell to a simpler, more embryonic, unspecialized form. Transdifferentiation (dotted arrows) occurs when an already differentiated stem cell forms cells outside its already established differentiation (extremely rare). Transdifferentiation is also referred to when a non-stem cell transforms into a different type of cell, which might actually take place through dedifferentiation and subsequent differentiation

2. Types of cell communication during co-culture

Cellular communication between cells in the human and animal body occurs via a variety of signalling pathways, which include endocrine signalling (via the blood stream), synaptic signalling (via nerve innervations), paracrine or autocrine signalling (signals released by one cell bind to membrane receptors of other cells), juxtacrine signalling (signals exposed on the membrane of one cell are bound by a membrane receptor of another cell) or gap junctional communication (intracellular signal exchange). When different cells types are co-cultured, they can interact and communicate via several different pathways, depending on their proximity and mutual ability to interact or communicate. The next section describes in more detail cell–cell signalling pathways reported to occur during co-culture of multiple cell types.

Gap junctional communication during co-culture has been shown to occur between cells from different cell types (heterotypic) but also between cells from different

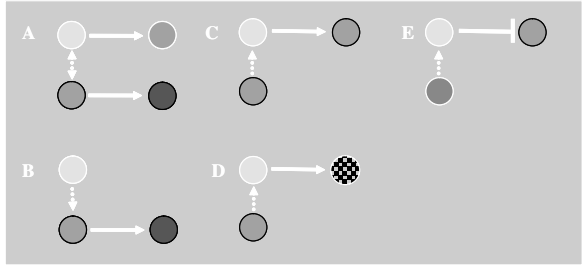


Figure 3. Cell behaviours described in co-culture studies in Table 1 are distinguished as follows: (A) cell types support physiology or differentiation of one another; (B) cell type 1 supports the differentiation or physiology of cell type 2; (C) one cell type (trans)differentiates towards the lineage of the cell type with which it is co-cultured; (D) one cell type differentiates into a tissue-specific lineage which is different from the cell that initiates or enhances the differentiation; (E) cell type 1 inhibits terminal differentiation of cell type 2

species (heterologous) (Lawrence *et al*., 1978; Stagg *et al*., 2006). While for gap junctional or juxtacrine communication cell–cell contact is required, for paracrine signalling close proximity alone is sufficient for the transfer of signalling molecules (Figure 1). Besides this,

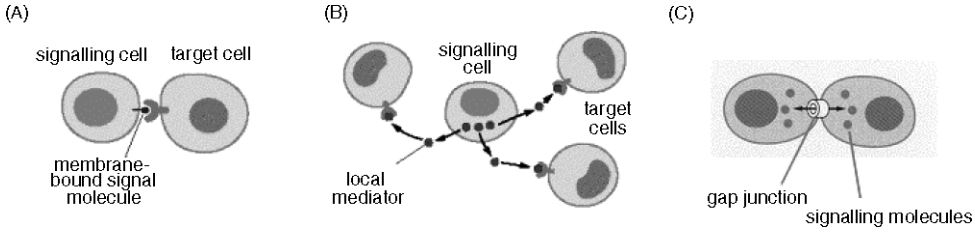


Figure 1. Forms of intercellular signalling during co-culture. (A) Contact-dependent signalling requires cells to be in direct membrane–membrane contact (juxtacrine). (B) Paracrine signalling depends on signals that are released into the extracellular space and act locally on neighbouring cells. (C) cells connected intracellular by gap junctions share small molecules and can therefore respond to extracellular signals in a coordinated way. Adapted from Alberts *et al*. (2002)

DOI: 10.1002/term

gap junctional communication enables exchange of intracellular signals via a direct connection between the cytoplasm of two cells. Paracrine or juxtacrine signalling involves extracellular secretion of signalling factors (Figure 1) (Alberts *et al.*, 2002).

The studies described in Table 1 emphasize that, during co-culture, cells from different tissues and even different species can communicate via paracrine, juxtacrine or gap-junctional signalling pathways. No correlation was found between the communication pathway of cell types in co-culture and the developmental stage of the donor of these cells (embryonic vs. immature vs. mature) or the influence they have on each other's physiology or differentiation status. At the same time, it was clear from some experiments that cellular interactions between different cell types in co-culture contributed to their tissue formation *in vitro*. Understanding which cellular communication pathways are important for tissue formation, and how the cellular interactions are regulated, could contribute significantly to the engineering of specific tissues *in vitro*, or development of therapies to stimulate tissue repair *in situ*.

3. Co-culture in tissue engineering

Tissues are complex three-dimensional structures with a highly organized architecture made up of cells and matrix. To maintain their form and function, the cells and matrix in a tissue are continuously interacting with each other and with cells in their surrounding tissues. Interactions of cells with their surrounding cells and matrix are important for a successful repair reaction. In tissue engineering, significant progress in guiding cell differentiation with growth factors, scaffold materials and architecture or extracellular matrix proteins has been made. However, in order to develop functional multicellular repair tissues, a number of challenges still need to be addressed. One challenge is to direct cellular interactions of multiple cell types involved in engineering repair tissues outside the body or initiate an appropriate repair reaction *in situ*.

In tissue engineering, co-culture was introduced to study the role of cell–cell communication and interactions between different cell types. Cell–cell interactions are important in tissue and organ development and they influence cell proliferation, differentiation and physiology (Bhatia *et al.*, 1997; Gerstenfeld *et al.*, 2003). In general, in tissue engineering co-culture was applied for two reasons: either two cell types were co-cultured to enhance tissue formation of a specific lineage; and/or two cell types were co-cultured to form a multicellular tissue or organ replacement and were expected to maintain or even support each other's specific lineages. To examine under which co-culture conditions either one cell type differentiates into the lineage of a second cell type, or when they maintain their respective lineages but reciprocally support each other's physiology or differentiation, is therefore of particular importance for tissue engineering.

In Table 1 we compare different parameters (origin of species of cell types, whether or not there is direct cell–cell contact, developmental stage of cell types) and the influence these might have on the cellular physiology and/or differentiation of several different cell types in co-culture. Co-culture of differentiated cells with a second differentiated cell type in most cases influenced the lineage-specific physiology of both cell types (Table 1). For example, when primary immature chondrocytes were co-cultured with primary immature osteoblasts, more extensive cell growth and matrix deposition was observed than in the chondrocytes control (Spalazzi *et al.*, 2003). In contrast, another study showed that when primary immature chondrocytes were co-cultured with primary immature osteoblasts, glycosaminoglycan deposition was significantly reduced (Jiang *et al.*, 2005). Both of these studies also showed that the two cell types in co-culture each maintained their specific cell phenotypes, rather than becoming transdifferentiated into the lineage of the other cell type.

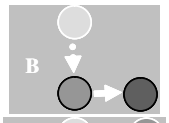
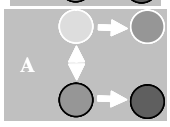
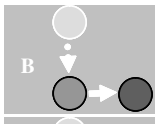
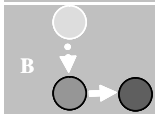
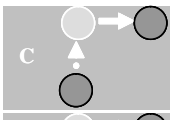
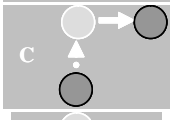
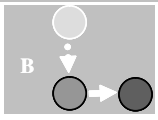
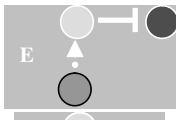
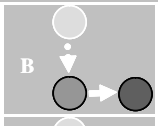
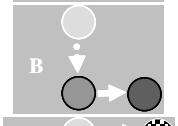
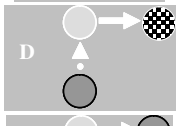
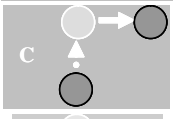
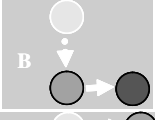
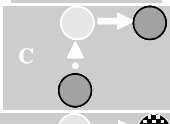
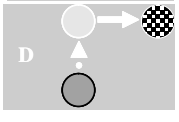
Further conclusions on the correlations between different co-culture parameters and their influence on cell physiology or differentiation of the multiple cell types in co-culture were limited. No correlation was found between the specific cellular interactions between cell types in co-culture and either their developmental stage, their differentiation stage or their different species of origin. For example, differentiation is reported to occur when either embryonic stem cells or mature stem cells were co-cultured with differentiated cells, regardless of whether or not cell–cell contact was established (Yoon *et al.*, 2005; Mummery *et al.*, 2003; Fukuhara *et al.*, 2003; Ravens, 2006; Buttery *et al.*, 2001). Mature mesenchymal stem cells do not differentiate when co-cultured in conditioned medium or co-cultured with immature differentiated cells without cell–cell contact (Yoon *et al.*, 2005). In contrast, other studies have reported that differentiation was initiated during co-culture when cell types were separated by means of a membrane insert (Buttery *et al.*, 2001; Gerstenfeld *et al.*, 2003; Yoon *et al.*, 2005). In conclusion, while cell–cell contact in some co-culture experiments seems to be crucial for cellular interactions, in other experiments co-culture in the same medium without cell–cell contact is sufficient. Although chondrocytes are the only cells residing in adult cartilage tissue, during cartilage (re)generation cellular interactions between prechondroblasts and other cell types is expected to determine the fate of chondrogenic cells.

4. Co-culture in cartilage research

The cellular interactions of chondrocytes *in vivo* depend on their locations in the cartilage zones. The spatial organization of articular cartilage recognizes four distinct zones, the superficial, transitional, radial and tight zones. Chondrocytes committed to these distinctive zones maintain their differentiated status through specific

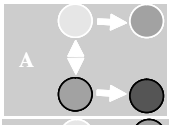
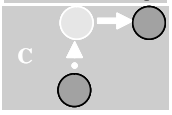
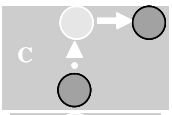
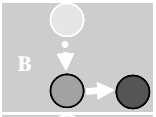
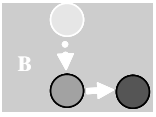
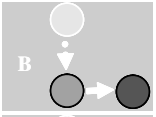
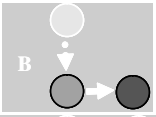
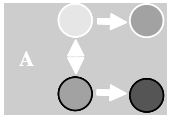
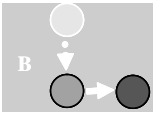
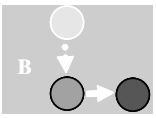
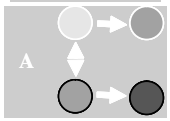
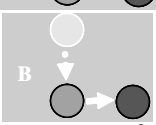
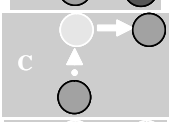
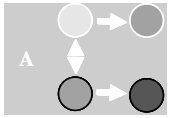
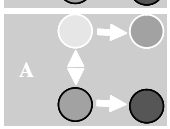
DOI: 10.1002/term

Table 1. Influence of co-culture on cell behaviour of different cell types when either in direct contact or not (for further explanation of the co-culture effect see Figure 3)

Tissue	Cell type 1	Cell type 2	Culture system	Co-culture effect	Reference
Bladder	Embryonic bladder smooth muscle (rat)	Embryonic bladder epithelium (rat)	Cell–cell contact		(Liu *et al*., 2000)
Bladder	Primary mature smooth muscle cells (bovine)	Primary mature urothelial cells (bovine)	Unclear		(Zhang *et al*., 2000)
Blood vessels	Immature expanded endothelial cells (bovine)	Immature expanded smooth muscle cells (bovine)	Cell–cell contact		(Williams and Wick, 2004)
Blood vessels	Umbilical vein endothelial cells (human)	Mature primary fibroblasts (human)	Cell–cell contact		(Wenger *et al*., 2005)
Bone	Embryonic stem cells (murine)	Embryonic osteoblasts (murine)	No cell–cell contact		(Buttery *et al*., 2001)
Bone	Mature mesenchymal stem cell line (C3H10T½) (murine)	Immature endochondral chondrocytes (avian)	No cell–cell contact		(Gerstenfeld *et al*., 2003)
Bone	Umbilical vein endothelial cells (HUVEC) (human)	Mature osteoprogenitor cells (human)	Cell–cell contact		(Guillotin *et al*., 2004)
Cartilage	Immature primary articular chondrocytes (rabbit)	Immature primary growth plate chondrocytes (rabbit)	No cell–cell contact		(Jikko *et al*., 1999)
Cartilage	Embryonic Notochordal cells (canine)	Mature and immature nucleus pulposus cells (bovine)	No cell–cell contact		(Aguiar *et al*., 1999)
Cartilage	Immature Keratinocytes (human)	immature elastic chondrocytes (human)	Unclear		(Neovius and Kratz, 2003)
Cartilage	Mature bone marrow mesenchymal stem cells (sheep)	Mature synovial cells (sheep)	No cell–cell contact		(Chen *et al*., 2005)
Cartilage	Mature fat mesenchymal stem cells (rabbit)	Mature intervertebral disc tissue from nucleus pulposus			(Li *et al*., 2005)
Hart	Fibroblastic immature ventricular fraction (rat)	Immature cardiomyocyte (rat)	Cell–cell contact		(van Luyn *et al*., 2002)
Hart	Mature mesenchymal stem cells (murine)	Mature cardiomyocytes (rat)			(Fukuhara *et al*., 2003)
Hart	Embryonic stem cells (human)	Mytomycin treated visceral endoderm like cells (embryonic cells)(murine)	Cell–cell contact		(Mummery *et al*., 2003)

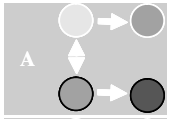
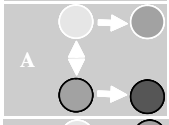
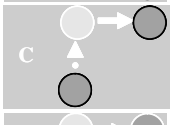
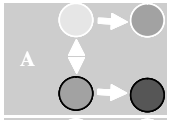
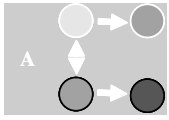
DOI: 10.1002/term

Table 1. (*Continued*)

Tissue	Cell type 1	Cell type 2	Culture system	Co-culture effect	Reference
Hart	Immature cardiomyocytes (murine)	Mature micro vascular endothelial cells (murine)	Cell–cell contact		(Narmoneva *et al*., 2004)
Hart	Mature mesenchymal stem cells (rat)	Immature cardiomyocytes (rat)	No cell–cell contact		(Yoon *et al*., 2005)
Hart	Mature bone marrow cells (murine)	Embryonic cardiac explants (avian)	Cell–cell contact		(Eisenberg *et al*., 2006)
Liver	NIH 3T3-J2 fibroblasts (murine)	Mature hepatocytes (rat)	Cell–cell contact		(Bhatia *et al*., 1998)
Liver	Mature sinusoidal liver cells (rat), liver epithelial cells (rat), 3T3 fibroblasts (murine), dermal fibroblasts (human) and oarta endothelial cells (bovine)	Mature hepatocytes (rat)	Cell–cell contact		(Goulet *et al*., 1988)
Liver	Mature bone marrow mesenchymal stem cells (female rat)	Mature hepatocytes (male rat)	Cell–cell contact		(Mizuguchi *et al*., 2001)
Liver	Mature aortic expanded endothelial cells (human)	Immature hepatocytes (rat)	Cell–cell contact		(Harimoto *et al*., 2002)
Liver	NIH 3T3 fibroblasts (mouse)	Primary mature hepatocytes (rat)	Cell–cell contact		(Kang *et al*., 2004)
Mucosa	Mature fibroblasts (human)	Mature keratinocytes (human)	Unclear		(Imaizumi *et al*., 2004)
Mucosa	Mature mesenchymal stem cells (human)	Mature respiratory epithelial cells (human)	No cell–cell contact		(Le Visage *et al*., 2004)
Muscle	Myoblasts cell line (C2C12) (murine)	Embryonic endothelial cells and HUVEC endothelial cells (human)	Cell–cell contact		(Levenberg *et al*., 2005)
Neuron	Mature fibroblasts and keratinocytes (human)	Embryonic dorsal root ganglia neurons (murine)	Cell–cell contact		(Gingras *et al*., 2003)
Neuron	Mature bone marrow mesenchymal stem cells (rat)	Mature Schwann cells (rat)	Cell–cell contact		(Zurita *et al*., 2005)
OC construct	Primary immature chondrocytes (bovine)	Expanded immature osteoblasts (bovine)	Cell–cell contact		(Spalazzi *et al*., 2003)
OC construct	Primary immature chondrocytes (bovine)	Primary immature osteoblasts (bovine)	Cell–cell contact		(Jiang *et al*., 2005)

DOI: 10.1002/term

Table 1. (*Continued*)

Tissue	Cell type 1	Cell type 2	Culture system	Co-culture effect	Reference
OC constructs	Immature epiphyseal chondrocytes (human)	Embryonic osteoblasts (human)	Cell–cell contact		(Mahmoudifar and Doran, 2005)
Retina	Mature endothelial cells (bovine)	Mature retinal cells (human)			(Dutt *et al*., 2003)
Neurosensory retina	Mature bone marrow stem cells (human)	Irradiated mature retinal epithelium cells (human)	Cell–cell contact		(Chiou *et al*., 2005)
Skin	Mature fibroblasts (human)	Mature keratinocytes (human)	Cell–cell contact		(Zacchi *et al*., 1998; Wang *et al*., 2003; El Ghalbzouri and Ponec, 2004)
Urethra	Mature fibroblasts and smooth muscle cells (human)	Mature urothelial cells (human)	Cell–cell contact		(Fossum *et al*., 2004)

cellular interactions. Chondrocytes in the superficial zone interact and maintain their phenotype and physiology through cellular interactions with synovial cells and factors present in the synovial fluid. Cellular interactions in the transitional zone and radial zone take place mainly between the chondrocytes and the extracellular matrix, or factors embedded in the extracellular matrix, whereas chondrocytes found in the tight zone at the cartilage–bone border also interact with osteoblasts from the subchondral bone and solid bone matrix.

In the last two decades, co-culture has been introduced in cartilage research (Goldring *et al*., 1984). Cartilage is a unique tissue in that it consists of only one cell type – chondrocytes. Cell–cell interactions between chondrocytes and other cell populations mainly take place at the border of cartilage. Therefore, in cartilage research co-culture has been used to study the development of osteoarthritis by looking at cellular interactions between articular chondrocytes and synovial cells (Goldring *et al*., 1984; Lubke *et al*., 2005; Wu *et al*., 2005) or chondrocytes and osteogenic cells (Jiang *et al*., 2005; Sanchez *et al*., 2005).

4.1. Co-culture of chondrocytes with synovial fibroblasts

Studies on interactions between chondrocytes and synovial fibroblasts have focused on the influence of rheumatoid arthritic synovial cells, cartilage homeostasis and neocartilage formation. Results from different studies showed that, while synovial cells from healthy tissue supported chondrogenesis of chondrocytes but also mesenchymal stem cells, synovial cells from a donor with rheumatoid arthritis invaded neocartilage (Lubke *et al*., 2005; Chen *et al*., 2005). More surprisingly, it was shown that, upon co-culture, heterologous rabbit chondrocytes and human synovial cells communicated through both intercellular calcium signalling through the gap junctions and through ATP-mediated paracrine stimulation (D'Andrea and Vittur, 1996). Together these studies suggested that synovial cell–chondrocyte interactions seem to be more important for homeostasis than cartilage tissue formation, and showed that the co-culture system was a suitable model to study these interactions. Another cell type interacting with chondrocytes *in vivo* is osteoblasts from the underlying bone.

4.2. Co-culture of chondrocytes with osteoblasts

A cartilage defect proceeding to the underlying bone, or the blood supply to subchondral bone being obstructed, results in osteochondritis dissecans (OCD). In these cases not only cartilage but also the subchondral bone is affected, resulting in poor mechanical properties (Crawford and Safran, 2006). Consequently, treatment of these defects would preferably be with an osteochondral construct. Osteochondral constructs are engineered by co-culturing two cell types (chondrocytes and osteoblasts) or two tissue types (cartilaginous and bone-like tissue) in close proximity, mostly in bioreactors. However, the studies reviewed here have dissimilar outcomes and sometimes the conclusions that were drawn even seem to be contradictory.

In the study of Jiang *et al*. (2005), the amount of glycosaminoglycan deposited by primary immature chondrocytes alone in micromass culture was significantly higher than when the chondrocytes were co-cultured

DOI: 10.1002/term

with a layer of primary immature osteoblasts. In addition, cell-mediated mineralization of osteoblasts alone was considerably higher than they were co-cultured with chondrocytes. Interactions between osteoblasts and chondrocytes apparently modulated their cell physiology. In contrast with the results of Jiang *et al*., a study by Spalazzi *et al*. (2003) showed that chondrocytes cultured on polylactide-co-glycolide with 45S5 bioactive glass scaffolds (PLAGA–BG) maintained their phenotypic morphology for a longer time in the presence of osteoblasts compared to a chondrocytes-only control. Moreover, more extensive matrix production and cell growth was observed in the co-culture group combining chondrocytes with osteoblasts, compared to the chondrocyte-only and osteoblast-only control groups, respectively. These differences in outcome are possibly explained by differences in the culture systems used. Micromass culture is known to support chondrogenesis while the layer of osteoblast cells seeded on top apparently is not likely to undergo osteogenesis (Jiang *et al*., 2005). In contrast, the PLAGA–BG composite, initially seeded with osteoblasts and subsequently with chondrocytes, seems to support osteogenesis rather than chondrogenesis (Spalazzi *et al*., 2003).

Finally, mature osteoarthritic articular chondrocytes were co-cultured with subchondral osteoblasts isolated from sclerotic and non-sclerotic areas of the underlying bone. The findings suggested that sclerotic osteoarthritic osteoblasts could initiate a chondrocyte phenotype shift towards hypertrophic differentiation, although the chondrocytes did not express type I or X collagen or alkaline phosphatase (Sanchez *et al*., 2005). The latter finding confirms, on a cellular level, what is already known from clinical practice. It emphasizes that, certainly for the treatment of osteochondritis dissecans but perhaps also for many other chondral defects, cartilage as well as underlying bone should be treated or replaced.

4.3. Co-culture in cartilage tissue engineering

When a defect is inflicted on cartilage, the lack of blood vessels and nerves results in the lack of a suitable cartilage repair or regeneration reaction. One challenge in cartilage tissue engineering is to direct the cellular interactions involved in either engineering a repair tissue outside of the body or an appropriate repair reaction *in situ*. Cellular (inter)actions can be influenced indirectly via scaffolds, or directly by supplying the cells with growth factors, extracellular matrix proteins or other cells. Articular cartilage is a resilient load-bearing tissue that provides a smooth, almost frictionless, surface to the distal ends of bones in synovial joints. The extracellular matrix is provided and maintained by the only cell type residing in cartilage, the chondrocytes, which are scattered through this matrix and constitute 5% of its wet weight.

In cartilage tissue engineering, attempts are made to overcome this lack of regeneration capacity *in vivo* by regenerating cartilage *in vitro* using the patient's own cells. It is very unlikely that complete cartilage regeneration is initiated by the delivery of one growth factor applied at a non-specific (over)dose at one time point or during a specific duration of time, or that delivery of one (or two) growth factors to a defect can trigger the whole cascade of cellular events necessary for regeneration into normally shaped and functional cartilage tissue. A whole range of factors exchanged during co-culture of two or more cell types might be involved in tissue regeneration.

The few co-culture experiments performed in cartilagenous tissue engineering focused mainly on generating nucleus pulposus tissue. Mesenchymal stem cells originating from bone marrow or fat tissue were co-cultured with nucleus pulposus cells. Upon co-culture, the expression of nucleus-specific markers Sox-9, aggrecan and collagen type II increased significantly in both mesenchymal stem cells and nucleus pulposus cells. These results suggested that paracrine signalling between the two cell types initiated chondrogenic stem cell differentiation (Li *et al*., 2005).

For another study on the influence of co-culture on inhibited terminal differentiation of articular chondrocytes, immature articular chondrocytes were combined with immature growth plate chondrocytes (Jikko *et al*., 1999). When co-cultured in a trans-well insert system, and hence without direct cell–cell contact, articular chondrocytes inhibited the terminal differentiation of growth plate chondrocytes. In contrast, medium conditioned by articular chondrocytes could not prevent the terminal differentiation of growth plate chondrocytes (Jikko *et al*., 1999). Apparently, paracrine signalling was not involved in the cellular interactions between articular chondrocytes and growth plate chondrocytes that are involved in regulating the terminal differentiation of the former.

Finally, Tsuchiya *et al*. (2004) were the first to co-culture expanded articular chondrocytes with bone marrow mesenchymal stem cells, allowing cell–cell contact. The results showed elevated safranin O staining with increasing initial percentages of expanded chondrocytes. Nevertheless, in our understanding, in the Tsuchiya study pellets were cultured in medium containing dexamethasone and TGFβ3. These growth factors in themselves might be responsible for differentiation of the expanded chondrocytes present in the pellets.

In conclusion, co-culture has proved to be a powerful tool in tissue engineering, not only to generate tissues and organs consisting of multiple cell types but also to guide and support the tissue formation of cartilage through cellular interactions with other cell types. The findings in co-cultured studies summarized in this review emphasize the importance of examining cellular interactions for engineering tissues with multiple cell types, but maybe surprisingly also for tissues containing only one cell type, such as cartilage.

DOI: 10.1002/term

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DOI: 10.1002/term