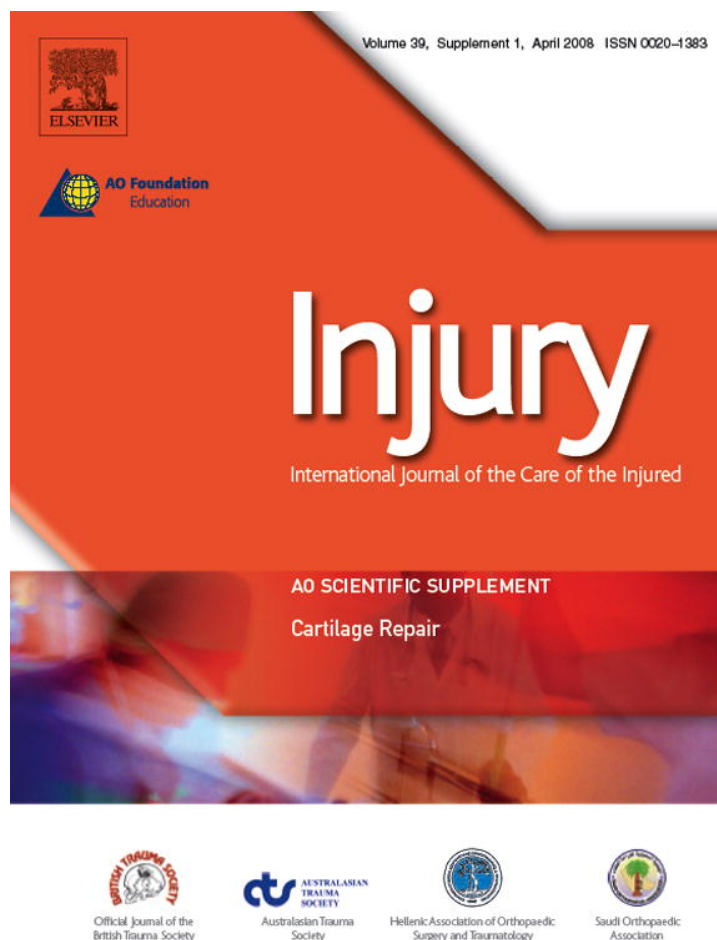


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# Tissue engineering of osteochondral constructs *in vitro* using bioreactors

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## KEYWORDS:

Mechanical stimulation, perfusion, articular cartilage, chondrocyte, osteocyte, bone marrow stromal cell.

**Summary**<sup>1</sup> Articular cartilage is a relatively simple tissue, but has a limited capacity of restoration. Tissue engineering is a promising field that seeks to accomplish the *in vitro* generation of complex, functional, 3-dimensional tissues. Various cell types and scaffolds have been tested for these purposes. The results of tissue engineered cartilage and bone are as yet inferior to native tissue. Strain and perfusion have been shown to stimulate cell proliferation and differentiation of various cell phenotypes. The perfect protocol to produce articular cartilage has not been defined yet. Bioreactors could provide the environment to engineer osteochondral constructs *in vitro* and to provide a stress protocol. The bioreactor has to provide an economically viable approach to automated manufacture of functional grafts under clinical aspects. Composite engineered tissues, like an engineered joint, represent a future goal. Cross-disciplinary approaches are necessary in order to succeed in engineering osteochondral grafts that provide adequate primary biomechanical stability and incorporate rapidly *in vivo* with histological appearance close to healthy osteochondral tissue. This review surveys current clinical and experimental concepts and discusses challenges and future expectations in this advancing field of regenerative medicine focusing human osteochondral constructs in bioreactors.

## State of the field

Articular surface or lost bone, when damaged by disease or trauma, has a size dependent limited regenerative capacity. Even so long bone defects have capacity to heal up to a critical size the situation is different and inferior for articular defects [66].

As early as 1743, Hunter [33] observed that cartilage, “once destroyed, is not repaired”. That premise has remained essentially unchanged for most of the subsequent 260 years. It has been reinforced by numerous studies documenting the abortive and fruitless response of chondrocytes to injury. Although the current disability and economic

burden of arthritis is substantial, the future burden will increase considerably with the increasing age of the population. Joint cartilage injury remains a major problem in orthopaedics with more than 500,000 cartilage repair procedures performed annually in the United States at a cost of hundreds of millions of dollars [31].

No consistently reliable procedure to regenerate joint cartilage currently exists. The breakdown of the articular surface is the beginning of degenerative osteoarthritis and it is followed by synovial irritation, remodelling of cartilage, disorganisation of soft tissue and eburnation of bone and cyst formation. The principal features of clinical presentation are pain, loss of movement and altered function.

Different treatment choices are open for these lesions and multiple surgical and nonsurgical procedures have been described [17]. When operative

<sup>1</sup> Abstracts in German, French, Italian, Spanish, Japanese, and Russian are printed at the end of this supplement.

intervention is chosen, various surgical techniques may be used: reconstruction or stimulation of biological regeneration of the joint surface formed by cartilage is the primary task prior to artificial joint replacement. Size dependent repair is recommended: For small defects (< 2 cm<sup>2</sup>), bone marrow stimulating techniques like microfracturing are recommended [20]. The microfracture technique was introduced by Steadman et al [69] and it opens access to the subchondral bone, bringing bone-marrow-derived progenitor cells to the articular surface. Clinical results demonstrate fibrocartilage formation and limited-sized defects can be treated satisfactory in more than 80% of cases. Of all the different approaches, only autologous osteochondral mosaicplasty has the ability to deliver genuine hyaline cartilage [27]. However, this technique is hampered by donor site morbidity and limited donor area. Gaps between implants and incongruity remain unsolved problems.

First-generation tissue-engineering procedures that are clinically applicable, such as autologous chondrocyte transplantation (ACT), follow a step-wise protocol with *in vitro* multiplication and implantation of the cells underneath a membrane. The earliest technique has been introduced by Brittberg et al in 1994 [6, 59] using amplified chondrocytes and a periosteum patch. Histological appearance of the neo-cartilage has been at best described “chondrocyte-like cells” despite good functional results for the patients. Calcification and fibrocartilage degeneration has been observed [40]. Lately, progenitor cells like bone marrow stromal cells (BMSC) have been differentiated into chondrocytes [11, 83]. More recent developments such as matrix-associated ACT are suitable for the largest defects [22].

Today, various cell-seeded constructs are commercially available to reconstruct cartilage defects (an overview is provided in Table 1). These matrix-associated techniques represent the second generation of clinically established tissue-engineered techniques. The application is limited to isolated defects of one side of the joint and some areas of the joints are inaccessible to the technique. Involvement of the subchondral bone is a further limitation. If there is no healthy cartilage surrounding the defect, use of an additional membrane is advocated and the outcome is limited. Affection of the subchondral bone is a further limitation. Clinical results of different cartilage repair techniques have shown to be somewhat disappointing in terms of life activity scores [40].

To demonstrate the superiority of any technique, better long-term clinical results than that obtained by natural history and current methods of treatment have to be proven. Long-term results for these

procedures may mean 20–30 years of follow-up. As several matrix associated chondrocyte transplantation techniques have developed recently, there are no long-term results available yet.

Experimental tissue engineering of autologous osteochondral composites *in vitro* is a promising therapeutic approach. These components can be manufactured as described above using a pre-defined matrix, eg, porous biomaterials seeded with the patients' own cells. Such grafts can either be generated by two independently cultured components or as one construct.

At present, there is no clinical application of tissue engineering techniques using the promising approach of progenitor cells in the field of an osteochondral transplant. Depending on the site of implantation, some biomechanical requirements have to be fulfilled: compressive stiffness, toughness, strength, resilience and shock absorption are characteristics of joints; toughness, strength and torsion stiffness are desirable, too. Bioreactors are established for cultivating different cell types under monitored and well-defined conditions and used to standardise several biological and engineering aspects, eg, safety, reproducibility and reliability.

This article reviews concurrent strategies and the state of the field in the literature regarding bone and cartilage engineering using progenitor cells, focusing on bioreactor techniques that stimulate cell growth and differentiation of osteochondral constructs *in vitro*.

## Tissue-engineering paradigms

As a simple tissue engineering technique in clinic, autologous spongiosa from the iliac crest is the gold standard for many orthopaedic procedures involving osteochondral defects. However, this technique is connected with more than 30% donor-site morbidity [28]. In the future, tissue-engineering techniques may allow joint reconstruction using autologous cells. The term “tissue engineering” in medicine was used at scientific conferences in the late 1980s [29]. Under the tissue engineering paradigms cells, biomaterial scaffolds and bioreactors are required [61]. Current engineering constructs of articular surfaces are as yet inferior to native articular cartilage. Cartilage can be characterised biomechanically as an isotropic material with no interstitial fluid flow during instantaneous and equilibrium conditions. The sponge effect describes a fluid shift when cartilage receives load. This allows the tissue to be a shock absorber. The cells are embedded within an extracellular matrix (ECM) and are responsible for

its production and maintenance. It has to be the aim in the tissue engineering process to mimic this tissue and its characteristics as good as possible. Multiple studies were performed and used articular chondrocytes [57], osteoblasts [38] and bone marrow-derived precursor cells [3]. After biopsy of cartilage tissue and isolation of the chondrocytes by enzymatic digestion of the ECM, the cells can be transferred in a culture flask. The cells proliferate with a doubling time of approximately 2–3 days and an amplification of more than 1000 times can be achieved within regular cell-culture media supplemented with growth factors. In close contact to each other or with several biomaterials, the cells no longer proliferate but start to produce typical ECM containing glycosaminoglycan and collagen II structured in a more or less characteristic manner for hyaline cartilage [74].

The application of mechanical stress has been proven to effectively enhance chondrogenic commitment when using progenitor cells [2]. Scaffolds have been seeded with cells statically and dynamically in stirred flasks, using hydrogel as a cell delivery vehicle or by medium perfusion. Bioreactors used for the cultivation are of multiple technical origins to try to simulate *in vitro* the superior bioreactor of the

human being [76]. The aim after transplantation is to achieve a fast and tight contact between the carrier and the bone or the cartilage. The requirements are even higher for an osteochondral construct as bone and cartilage should be transplanted as a single construct. Osteochondral lesions are mostly treated like isolated cartilage defects. However, a viable osseous sublayer is necessary to provide a stable basis for incorporation.

### Cell resources

Potential cell resources for tissue-engineered osteochondral grafts are: cartilage autograft, cartilage allograft (banked), bone autograft, bone allograft, perichondrium, periosteum, induced skin fibroblasts, induced fat cells, bone marrow stroma or progenitors/precursors (stem cells).

Different biological preparations of cells have been proposed for use in osteochondral defect treatment. Primary cells that reside within bone or cartilage and maintain and remodel the tissue are initially ideal candidates, but they are limited in number and expansion leads in case of chondrogenic cells to de-differentiation or a loss of the cell phenotype.

Table 1: Existing second generation tissue engineering products for cartilage regeneration

Product	CaReS®	MACI®	Bioseed®	Novocart®	AMIC/ Chondro Gide®
Material	rat collagen I	bovine collagen I	polymer/ fibrin	biphasic collagen I	bovine collagen I/ III membrane
Culture duration (days)	14 (3-D)	14 (2-D) 7 (3-D)	10 (2-D) 7 (3-D)	8–9 (2-D) 8–9 (3-D)	0
Transplant size (mm)	unlimited	40–50	20x30	30x50	40x50
Transplant height (mm)	variable	1	1.5	1.5	variable
Cell number	< 10 x 10 <sup>6</sup>	~ 10 x 10 <sup>6</sup>	~ 20 x 10 <sup>6</sup>	15 x 10 <sup>6</sup>	none
Fitting	trimming, inlay	inlay	inlay	inlay	trimming, inlay
Fixation	fibrin glue/ membrane	fibrin glue	suture	suture/ none	fibrin glue
Arthroscopic application	no	no	no	no	no
Multicentre-study	unpublished	yes	yes	yes	no
Comments	suitable for limited arthritis	now available from Matricell®	polymer content < 10%	navigated application possible	
Price (Euro)	5000	5500	6000	5000	5000
Web site	www.arthrokinetics.com	www.verigen.com	www.biotissue-tec.com	www.tetec-ag.de	www.geistlich.ch

Next to proliferation of adult, differentiated cell lines of bone and cartilage, human bone marrow stromal cells (hBMSC) are considered the premier source for tissue engineering. BMSC can be obtained easily from various locations and amplified *in vitro* [12]. These pluripotent stromal stem cells from bone marrow can be isolated and cultured *ex vivo* and then their histogenic differentiation can be induced by external factors [68]. The number of cells available can be increased by  $10^6$  times and moreover, they can be frozen and thawed without losing their ability to proliferate and differentiate into bone [45], cartilage [4], and fibrous tissue [1]; the process is enhanced by individual cell-cultivation protocols.

Many authors have used differentiated or multipotent cells for tissue engineering [7, 10, 51, 59, 61]. Otherwise to date, the plasticity, multipotency and characteristics of progenitor cells from skeletal tissue remain poorly defined. Differentiation and immunophenotyping (CD 105+, CD 34-, 45-) has to determine the potential of these cells as an unique alternative model and cell source for restoring damaged tissue. This technique is clinically established as the microfracture or bone-marrow-stimulation technique without any additional enhancement of the local cell population [69]. However it has not been proven that a special cell line is responsible for the generation of fibrous cartilage as a mixture of all bone marrow stromal cells is clotting in the defect. Another option in induction of cell differentiation next to growth factors which are discussed in one of the next sections is gene therapy. Different genes can be expressed to regulate cell mechanisms in order to reach a desired differentiation. It requires neither long-term transgene expression nor closely regulated levels of transgene expression. An increasing amount of evidence indicates that gene transfer can aid the repair of articular cartilage, menisci, intervertebral disks, ligaments and tendons [18]. Current problems *in vitro* cell culture of BMSC are the long term phenotype, ossification of transplants and the limited cell number (max.  $10^6$  BMSC in a bone marrow puncture). Long-term differentiation is a key issue as malignant transformations have been observed [85]. Better-defined differentiation remains a desirable aim. Mechanical stress has been demonstrated to stimulate the secretion of osteogenic [38] and chondrogenic proteins in BMSCs supporting their differentiation [4].

Recent studies have identified the presence of an abundant source of stem cells in subcutaneous adipose tissue. These cells, termed adipose-derived adult stem (ADAS) cells, show characteristics of multipotent adult stem cells similar to those of BMSCs and, under appropriate culture conditions, they synthesise cartilage-specific matrix proteins

that are assembled in a cartilaginous or osseous extracellular matrix. The growth and chondrogenic or osteogenic differentiation of ADAS cells is strongly influenced by factors in the biochemical as well as biophysical environment of the cells [26,80].

Last but not least, embryonic stem cells (ES) offer great potential in tissue engineering, although ethical concerns exist. These pluripotent cells with the ability to differentiate into multiple tissues, derive from the inner cell mass of blastocyst stage embryos [39]. Chondrogenic differentiation requires a passage through the embryonic body stage. Polymers that can resist cell-induced contraction have been used in combination with ES [46]. The disadvantage of ES is the formation of teratomas in joints [78]. ES cell expansion requires laborious culture conditions, specifically the need for feeder layers, and therefore still poses difficulties for the large-scale expansion needed for therapeutic applications.

Whatever cell source is selected seeding those on the scaffold is an important step in the tissue-engineering process. High initial cell densities have been associated with enhanced tissue formation, including cartilage matrix production and bone mineralisation [19, 30]. A homogenous cell distribution is also desired, too [52]. The use of biomaterials for osteochondral defect treatment seeded with autologous stem cells appears to be useful as the *in vitro* chondrogenic pre-differentiated cells tend to dedifferentiate without their natural 3-D matrix surrounding [35].

### Scaffold selection in tissue engineering of osteochondral constructs

Potential scaffolds for the tissue engineering of bone or cartilage include:

#### Biologic materials

- perichondrium
- SIS (small intestinal submucosa)
- collagen (I) sponge
- collagen (I)-GAG (glycosaminoglykan)
- collagen (II)-GAG
- collagen (III)-GAG
- fibrin
- hyaluronan
- gelatine
- alginate
- agarose
- chitosan
- periost
- DBM (demineralized bone matrix)
- allogeneic/xenogenic bone

### Synthetic materials

- PL (Polylactic acid)
- PGLA (Polyglycolic acid and copolymers)
- CF-PU-PLLA (Carbonfibre-Polyurethane-Poly (L-lactide)-Graft)
- CF-Polyester (Polyester-Carbonfibre)
- PU (Polyurethane)
- PLLA (Caprolactone (Poly-L-Lactide/epsilon-Caprolactone)
- PLLA-PPD (Poly- L-Lactic Acid and Poly- p-Dioxanol)
- PVA-H (Polyvinylalcohol-Hydrogel)
- $\beta$ -TCP (Tricalcium phosphate)
- CDHA (Calcium-deficient hydroxyapatite)

For the 3-dimensional culture of cells, both synthetic [51] and biologic scaffolds [64] have been suggested as carriers. Desired features are a controlled biodegradability, suitable mechanical strength, ability to be processed in different shapes and sizes, and ability to regulate cellular activities, such as proliferation and differentiation via a defined surface chemistry [73]. Recently tested scaffolds were porous biodegradable synthetic polymers [51], benzylated hyaluronan [58], porous collagen, [75] and porous silk [76]. Ceramics like hydroxyapatite or  $\beta$ -tricalciumphosphate consist of minerals of the natural bone matrix. These scaffolds are by nature brittle, and are only partially replaced over time [24, 25]. Animal studies indicated this major problem: the long term fate of these scaffolds. This is a major shortcoming of tissue engineered osteochondral grafts. The 3-dimensional shape of scaffolds can be customized. Some authors have used synthetic scaffolds made out of polyglycolide (PGA) and/ or polylactide (PLA) for the 3-dimensional culture of BMSC [51]. Degradation time could be controlled by the amount of polylactide within the scaffold. Pure PGA/PLA constructs may evoke inflammatory reactions and osteolysis [71]. Growth factors could be added in order to increase the osteogenic potency of these materials and are discussed in the next section.

Biological materials such as bovine (xenogen) or human (allogen) cancellous bone offer good osteoinductive and biomechanical properties. DBM contains growth factors and promotes osteogenic differentiation and has already been used clinically [63,64]. Different collagen matrices (I, II, III) are clinically and experimental established (compare Table 1). To our knowledge no special biomaterials for osteochondral defects are in standardised clinical use. Such lesions are mostly treated as single cartilage defects. Alternatively, they are replaced by artificial, cell-free matrices, such as SaluCartilage™, polyvinyl-alcohol-hydrogel, CartiPlug® and

collagen I hydrogel (see Table 1) or cancellous bone matrices, eg, Tutobone® (Tutogen Medical GmbH, Neunkirchen a. Br., Germany).

Designing osteochondral tissue needs a specific strategy. The implant needs to copy the natural contours of the articulating surface, show adequate mechanical properties and have early functional load-bearing abilities. However, several new implant materials have been investigated in recent years to combine bone and cartilage defects [9, 21, 81]. Typically, the cartilage region is seeded with cells, either chondrocytes or BMSCs, while the bone region remains acellular or is seeded with osteogenic cells such as osteoblasts, periosteal cells, BMSCs or bone marrow. The minimum requirement for a scaffold is to provide a temporary structure while the cells seeded within it synthesise a natural environment parallel to the degradation of the matrix. Biodegradable polymer scaffolds have great potential for use as matrices. They can be mono- or biphasic containing natural products like collagen and hydroxylapatite or artificial polymers such as cross-linked hydrogels.

Various scaffold strategies for osteochondral defects, such as replacement without a cartilage layer, composite scaffolds, a heterogeneous or bilayered scaffold or a homogenous, single-layered scaffold, are discussed in the literature [50]. In most cases, biphasic scaffold materials are introduced in which two single materials (eg, calcium phosphates for the bony part and gels for the cartilage part) are manufactured separately and then stuck together, eg, using fibrin glue [51]. Due to high shear stress in the joint during movement most of these material combinations are likely to disintegrate. Secondary integration to the surrounding natural tissue is desired. Surgical options such as sutures or glue-like fibrin sealant are able to fill the gap on biological integration. However, there is no biomechanical investigation proving this hypothesis.

### Effects of culture conditions, bioreactors, mechanical stimulation and perfusion

Culture conditions are another key issue in tissue engineering. In a static culture, proliferation and differentiation can be performed using various growth factors, as mentioned above. Culture parameters are temperature, pH, pCO<sub>2</sub>, oxygen, pressure and media supply (such as growth factors), which have to be maintained within a defined range to ensure reproducibility and standardisation.

## Growth factors

Different media for bone and cartilage are required as different growth factors can be used, as described above. Those signalling molecules bind to cell receptors to activate a signal pathway that instructs cells to proliferate, differentiate and synthesise ECM proteins during tissue regeneration. Dexamethasone,  $\beta$ -glycerophosphate and ascorbic acids together comprise an osteoinductive medium which in a dose and time-dependent manner can effectively enhance the function of various growth-factors [8]. Dexamethasone enhances specific markers of osteoblastic differentiation in specific cells by decreasing collagenase expression, suggesting that endogenous collagenase may regulate osteoblastic differentiation of these cells. Growth factors found in bone that are potential chemokines include IGF (insulin like growth factor), PDGF (platelet-derived growth factor), FGF (fibroblast growth factor), and TGF- $\beta$  (transforming growth factor) [49, 83].

Chondrogenic differentiation can be performed in the micromass culture model or pellet culture adding recombinant TGF- $\beta$ , dexamethasone,  $\beta$ -glycerophosphat and ascorbic acid [41, 60] to the media for different time periods. Additional serum of the donor species is required to support proper nutrition of the cells in culture. The TGF- $\beta$  family is the most potent inducer of chondrogenesis in BMSCs [34, 44, 47, 70, 76].

The bone morphogenetic proteins (BMPs) present in DBM are members of the TGF- $\beta$  superfamily and some have chondrogenic properties [56]. Chemokines like different types of IGF, FGF and PDGF are known to promote various physiological parameters such as proliferation and they work positively together with the TGFs [70].

A recent trend in tissue engineering is to combine those growth factors to maximise their impact on *in vitro* proliferation and differentiation [34]. But heterogeneous results have been reported in the literature [37]. The primary task in applying those media additives is to optimise the desired tissue regeneration. Obviously, the requirements for cartilage and bone are different.

## Bioreactors

From beer brewing to tissue engineering, bioreactors bring advantages to creating products. Bioreactors can be used for cells and other microorganisms to produce various proteins or drugs. The basic concept of a bioreactor is to provide an environment that is advantageous to the creation

of a desired product, whether it is alcohol or ECM as in engineering of osteochondral constructs. Nutrients, waste, temperature, and gas levels must be carefully controlled. If these conditions are kept at an optimal level, then the reactors can be run successfully for long periods of time.

Manufacturing an osteochondral construct to be operated successfully in an automated bioreactor needs to include low cost state-of-the-art techniques for monitoring and controlling the physicochemical culture parameters. Conventional spinner flasks, rotating-vessels, concentric cylinders and bioreactors with different and/or combined stimulation procedure have been designed. The basic mechanical and/or hydrostatic or hydrodynamic forces when using a bioreactor are displayed in Fig. 1. Basic forces can be applied in static (gravity), hydrostatic (continuous fluid pressure) and mechanostatic systems (continuous pressure over a lever). Dynamic forces are applied via fluid flow, shear and an electric field or motor-driven devices. It is well known that these physical stimuli can modulate the metabolism of cartilage and bone, and when applied in special mixture with specific magnitudes and frequencies, may

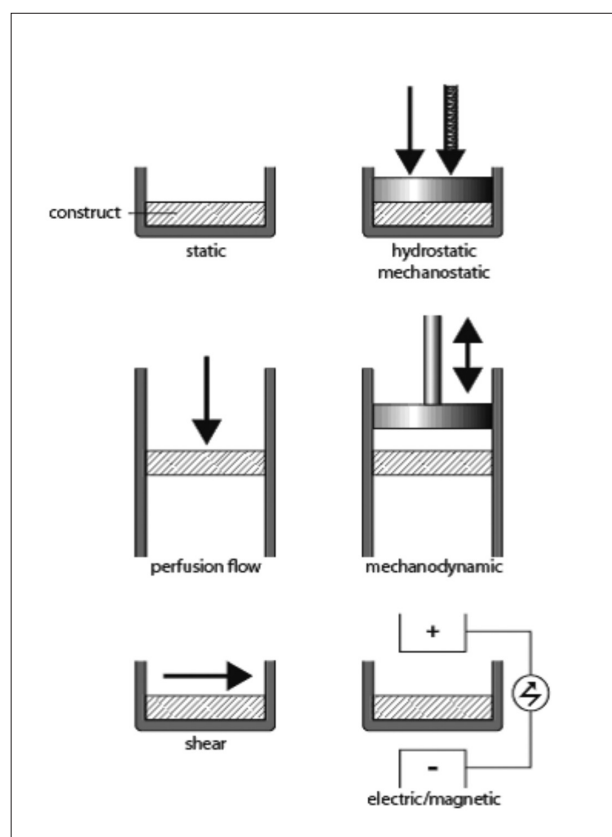


Fig. 1: Basic bioreactor concepts. Schematic overview of different stress protocols applicable to scaffold constructs.

upregulate the production of ECM components. Multiple static and dynamic systems have been designed and used. For cartilage engineering, hydrostatic application devices have been successfully used [2, 15]. Further developments introduced dynamic systems using rotation to foster cyclic stress patterns. For space biology, various rotating devices have been tested to mimic gravitation [79]. The next step within the development process was the introduction of continuous perfusion in vascular-graft tissue engineering [54]. Within the dynamic systems, perfusion and mechanical stress were used. Cyclic mechanical stretching modulated the secretion pattern of growth factors in human tendon fibroblasts [83, 84]. Cyclic hydrodynamic stimulation has been shown to enhance the chondrogenic commitment of hBMSC in a pellet culture [4, 14].

For cartilage-like constructs, hydrodynamic systems have been successfully applied using rotating reactors [72, 76]. There have been several investigations dedicated to examining the influence of cyclic mechanical stretching on osteoblasts obtained from cancellous bone chips [5, 38, 55]. Their analysis indicates that a strain rate of 0.1% (1000 microstrain) and a frequency of 1 Hz resulted in an increased proliferation (10–48%) and carboxyterminal collagen type I propeptide release (7–49%), while alkaline phosphatase activity and osteocalcin release were significantly reduced by 9–25% and 5–32%, respectively. In a 3-point bending reactor hBMSC osteogenic differentiation was enhanced [53]. The axial strain limit that caused an alignment response was lower for fibroblasts, 4.2 +/- 0.4%, than for osteoblasts, 6.4 +/- 0.6% [55].

Experiments that investigated the influence of cyclic strain on 3-dimensional cell cultures (BMSC in a collagen gel, [1]) show that a strain rate of 10% and 1 Hz significantly fostered cell alignment and density and upregulated collagen I and III expression. In this setup, there was no upregulation of bone or cartilage specific differentiation markers. Cyclical stretching (8% strain) and dexamethasone both enhance the osteogenic differentiation of human BMSC when combined [36]. The application of strain *in vivo* in fracture models indicates that low-magnitude, high-frequency mechanical signals can restore anabolic bone cell activity inhibited by disuse (0.1%, 20Hz; [62]). Other studies suggest that a strain magnitude of 10% is most effective in producing callus *in vivo* [82]. Mechanotransduction was postulated to affect the proliferation and cell synthesis directly. Continuous perfusion has been proven to stimulate cell proliferation and osseous differentiation of BMSC in a titanium mesh [5].

Cyclic hydrodynamic stimulation has been shown to enhance the chondrogenic commitment of hBMSC in a pellet culture [4, 14]. For cartilage like constructs hydrodynamic systems were successfully applied using rotating reactors [72, 76]. Mature chondrocytes of bovine origin were seeded on agarose hydrogels and stimulated in a perfusion reactor [32]. Cartilage like properties were reported over an 8-week stimulation protocol. Different protocols are described for perfusion patterns [13, 57, 65, 67].

Aggregates of BMSC that were exposed to cyclic hydrostatic pressure for 7 days reacted with a significant increase in collagen and proteoglycan content [4]. Optimization of high cell density in selected regions like the bone/cartilage interface of an PGA-scaffold coupled with the stress of a dynamic recirculation bioreactor has a significant influence on the quality of tissue-engineered cartilage when investigated ECM proteins like GAG and collagen [49]. Mechanical loading using dynamic compressive strain at 0.3, 1 and 3 Hz on chondrocytes seeded in an agarose gel. demonstrated an increased GAG content. [42,43]. Elder et al demonstrated an enhanced chondrogenic differentiation under cyclic mechanic compression (9.25 kPa, 0.33 Hz 2h for 3 days) of BMSCs in a agarose gel [16]. All studies within the physiological forces indicated under cyclic stress a positive influence upon the quality of the extracellular matrix [13, 23, 52, 77]. Magnitude, frequency, seeding density and duration of loading influences the outcome as recently was demonstrated in hybrid matrices to mimic osteochondral tissue [48]. Bioreactors could add mechanical strength and improve three dimensional structure of an osteochondral construct. Thus, the tissue could provide sufficient mechanical properties to be implanted. Despite the accumulating evidence that stimulation via different stress protocols very little is known about specific forces or ranges of application. The superior *in vitro* model providing the best stress protocol whether it is continuous or intermittent with the correct force magnitudes and frequencies has not been identified as yet. Although a sufficient level of stress, strain and/or shear seems to be essential to regenerate osteochondral tissue [77]. It could be hypothesized that different protocols for the cartilage and bone region of an osteochondral construct could be necessary (eg, perfusion of the bony part vs. compression of cartilage-like structures). Two chamber bioreactors could be advantageous, but even more complicated in practice. We were able to develop a system in which perfusion and mechanical stress can be transferred with different

frequencies upon different construct (fig. 2). The optimum stimulation protocol for an osteochondral construct has yet to be found.

## Conclusion

Articular cartilage is a relatively simple tissue because of its cellular homogeneity and avascularity. Composite engineered tissues, like an engineered joint, represent a future goal. Tissue engineering is clinically established for small volumes of tissue that are of limited 3-dimensional complexity. Mechanical stimulation and perfusion play a key role in functional repair as well as cell differentiation. The integration of mechanical stimulation in the tissue-engineering process may lead to progress in the structural and biomechanical properties of osteochondral tissues and offer new possibilities for managing joint injuries and degenerative diseases.

Cell selection, scaffold design and biological stimulation remain the key challenges of functional tissue engineering. Advances in materials design may generate smart scaffolds that will control tissue topology and have surface modifications to stimulate cell attachment, differentiation and growth. Within the next step, bioreactors have to prove their efficiency regarding controlling cell proliferation and differentiation under the influence of different stress protocols.

The perfect protocol to produce articular cartilage has not yet been defined. This is a challenging task to create applicable implants in the future. Key issues are clearly the use of compressive forces for

cartilage and sufficient perfusion for bone. Another key issue is a high cell density. Ethical problems have to be considered and prior industrial product certification (eg, GMP) has to be obtained. Country dependent health economic capacities and regulations have to be taken into account. Cross-disciplinary approaches are necessary to succeed in engineering osteochondral grafts that provide adequate primary biomechanical stability and incorporate rapidly in vivo with histological appearance close to that of healthy osteochondral tissue.

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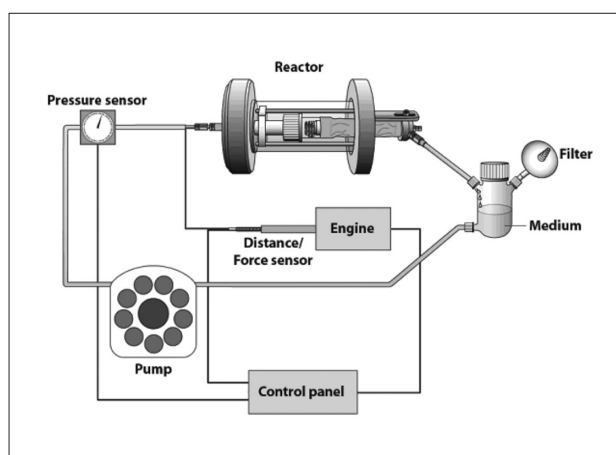


Fig.2: MHH bioreactor: The reactor contains a chamber that hosts the construct. A pump steers the flow of medium and an electric engine drives a gauge to provide cyclic mechanical loads. Every force is monitored and steered by a control panel on a personal computer.

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