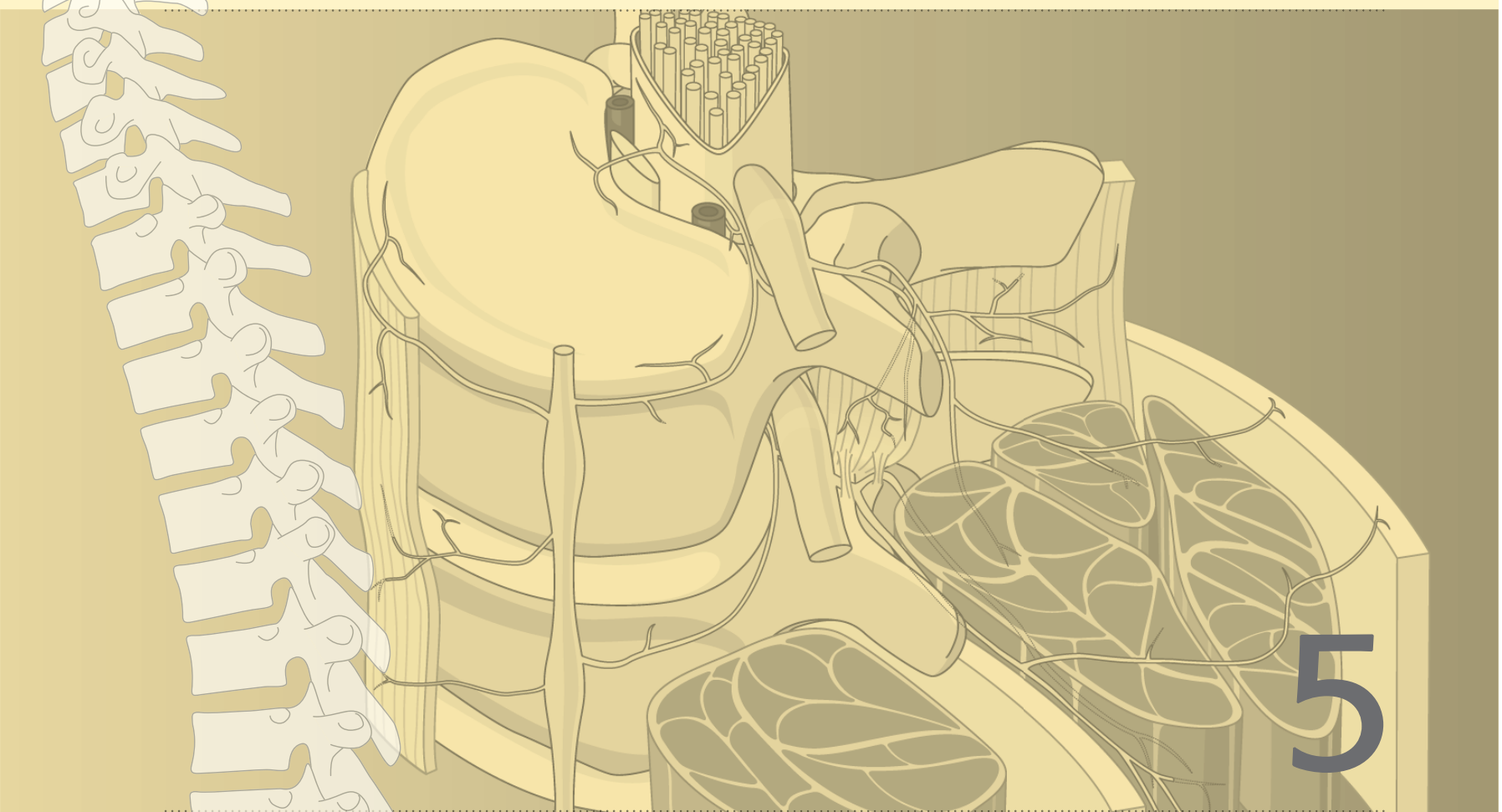
Norbert Boos

BIOLOGY OF THE SPINE



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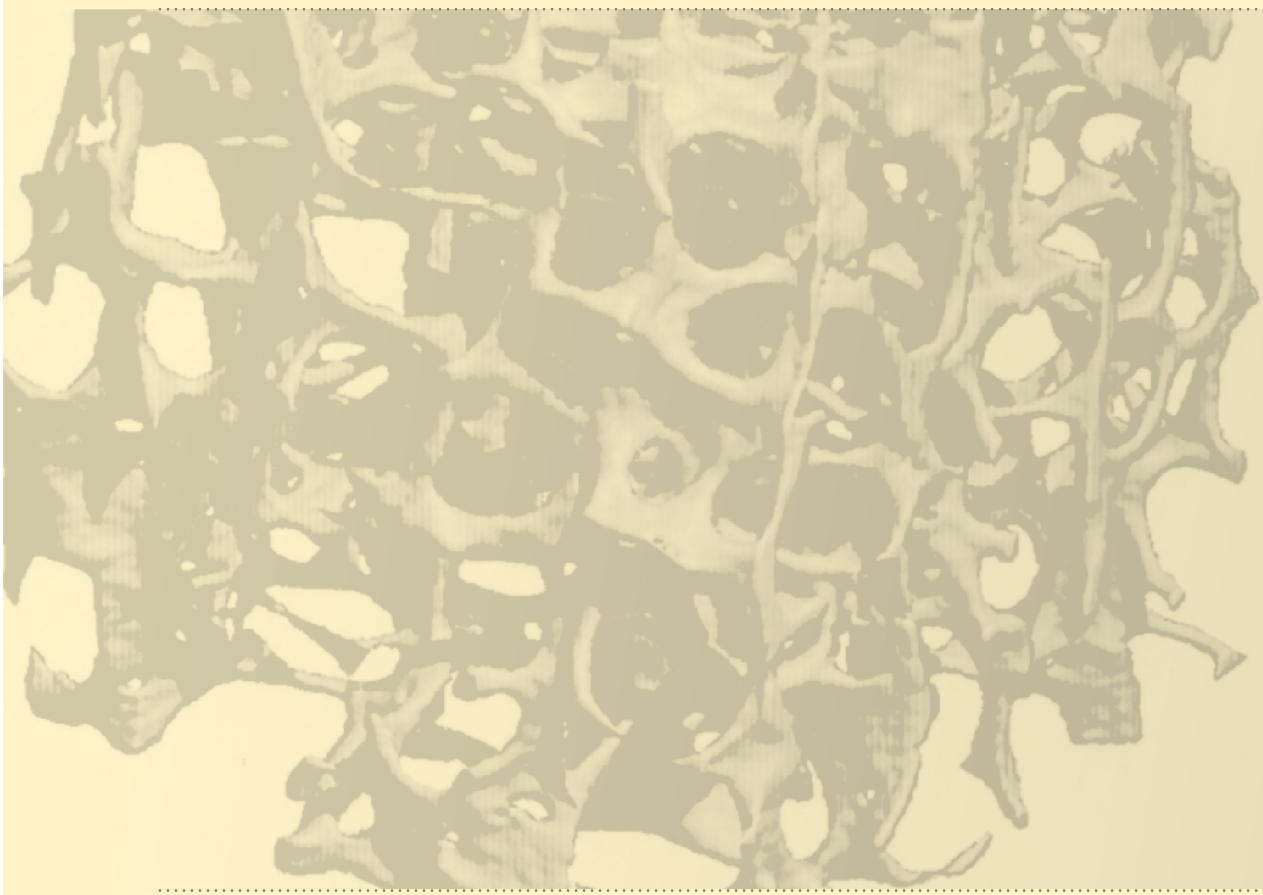

5 BIOLOGY OF THE SPINE

INTRODUCTION

In the past two decades, spine surgery has made enormous progress. This progress became possible with the advent of second and third generation spinal implants. These implants allowed for a short segmental and angle-stable fixation, correction of severe deformities, and facilitated postoperative treatment by early mobilization. Very recently motion-preserving implants, such as dynamic instrumentation systems, disc arthroplasty, and interspinous process implants, have added to the armamentarium of the spine surgeon. Vertebroplasty and kyphoplasty have come into widespread use for the treatment of osteoporotic fractures. Bone substitutes and cytokines are used even more frequently to achieve spinal fusions.

The biggest risk of these technology-driven developments is that we forget about the biology of the spine and basic biological principles. In this context, a disc prosthesis will ultimately fail if we do not understand that the surgery is addressing not only the intervertebral disc, but the whole motion segment including facet joints, muscles, and ligaments. Our understanding of the basic process leading to back pain is still very limited and we still have almost no clue about the underlying molecular mechanisms. The classic concept of fusion, ie, eliminating painful motion within segments is being increasingly challenged by the clinical success of motion-preserving implants. We still do not understand the weak correlation between morphological alterations of the intervertebral disc and pain. Similar tissue alterations can cause pain in one individual and remain asymptomatic in another. The molecular mechanisms of disc degeneration are still being unraveled, and the potential for a more biological repair remains unexplored. With the age of the population increasing in developed countries, osteoporosis and related fractures are becoming an increasing challenge for the spine specialist. Knowledge of baseline therapy and a potential prophylaxis of osteoporosis are necessary in dealing with

this pathology. The development of bone substitutes and bone morphogenetic proteins will substantially influence spine surgery in the near future.

The following chapters will provide an overview of biological principles related to the treatment of spinal disorders. The understanding of these biological principles is a prerequisite for the selection of appropriate treatment modalities. Some of the most fundamental biological principles are as simple as they are obvious, but clinical practice continuously demonstrates that they are being jeopardized.

- The spine is a complex organ consisting of a functional chain of motion segments. Surgery to one part also affects adjacent parts.
- The kinematics of the spinal motion segment and the intrinsic and extrinsic effect of the spinal muscles are very complex and cannot easily be mimicked by implants.
- Disc degeneration starts as early as the second decade of life and is related to age rather than pathology.
- The vast majority of disc alterations which are addressed by surgery exhibit only a week correlation to pain.
- Osteoporosis is a systemic disease and changing the stiffness in one segment (eg, by vertebroplasty) will have an impact on the adjacent segments.
- Cement augmentation does not solve the problem of the diseased bone.
- Fusion implants will eventually fail when solid fusion does not occur.
- Bone substitutes and cytokines cannot compensate for insufficient fusion techniques.

The spine specialist, and in particular the spine surgeon, must be aware of the aforementioned problems, challenges, and biological principles when choosing treatment modalities and caring for patients.

5 BIOLOGY OF THE SPINE
5.1 BIOLOGY OF THE MOTION SEGMENT

5 BIOLOGY OF THE SPINE

5.1 BIOLOGY OF THE MOTION SEGMENT

1 INTRODUCTION

The basic functions of the human spine are to support the body, protect the spinal cord and nerve roots, and allow for movements of the trunk. Thus, the spine simultaneously provides enough stability to keep an upright posture, but at the same time allows for enough mobility of the trunk. The spine can further absorb energy and thereby protect itself against impact. The spine consists of seven cervical vertebrae, twelve thoracic vertebrae, five lumbar vertebrae, five fused sacral vertebrae, and three to four partially fused vertebrae forming the coccyx. The unfused vertebrae are separated by the intervertebral discs in the front and the bilateral zygapophyseal joints (so-called facet joints) in the back.

The vertebrae are further connected by spinal ligaments, facet joint capsules, and segmental muscles. The spinal ligaments consist of interspinous, supraspinous intertransverse, yellow, anterior, and posterior longitudinal ligaments. In contrast to the extrinsic muscles, the intrinsic muscles span only two vertebrae and consist of splenius, erector spinae, transversospinal, and segmental muscles. The smallest anatomical unit of the spine, which exhibits the basic functional characteristics of the entire spine, is called motion segment or functional spinal unit (FSU) (**Fig 5.1-1**). The term motion segment was first coined by Schmorl and Junghanns in 1968 [1].

Normal spinal function largely depends on the integrity of these different components and their coordinated interplay. Any alteration of these components or their interplay may result in dysfunction finally leading to back pain, deformity, and neurological compromise. Kirkaldy-Willis introduced the term “the three-joint complex” to highlight the importance of normal interaction between the three joints (ie, intervertebral disc and facet joints) for a healthy spine. However, in this complex the role of the back muscles is not incorporated, although undoubtedly essential for the normal functioning of the motion segment.

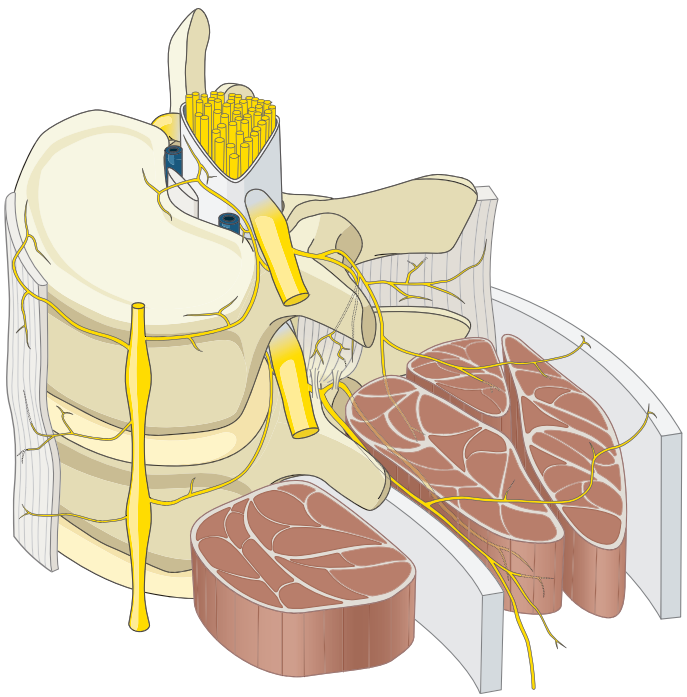

Fig 5.1-1
The functional spinal unit (FSU)

Our present understanding of the underlying mechanisms of back pain is still poor. Progress in the understanding of back pain and also of the development of spinal deformity depends on basic knowledge of the biology and the interplay of the functional spinal units from the level of gross anatomy to the level of cells and extracellular matrix components. While increasing knowledge has been gained on the biology of the intervertebral disc, little is known about the biology of facet joint dysfunction and its clinical relevance. The gross morphology of the spinal muscles and their basic kinematics has been extensively studied. However, only sparse information is available with regard to spinal muscle function on the level of the motion segment and its relation to the development of degenerative changes.

The objective of this chapter is to provide a short overview on the current concepts of the biology of the spinal motion segment which, however, is hampered by limited knowledge in this area.

2 INTERVERTEBRAL DISC

Since its first description by Mixter and Barr in 1934 [2], alterations of the intervertebral disc have been the focus of research exploring its association with back pain. Because of the importance of the intervertebral disc, the authors will separately cover biology in health and disease in chapter 5.2 Aging and pathological degeneration.

3 FACET JOINT DYSFUNCTION AND OSTEOARTHRITIS

The facet joints are also called zygapophyseal joints and resemble the features of synovial joints. They are an essential part of the posterior support structures of the spine consisting of: pedicles, lamina, and spinous and transverse processes. Adams and Hutton [3] found that the facet joints resist most of the intervertebral shear force. The posterior annulus is protected in torsion by the facet surfaces and in flexion by the capsular ligaments. The posterior elements also serve as anchors for the spinal muscles which stabilize the spinal column. The biomechanics of the functional motion segment is covered in chapter 4.1 General biomechanics of the spinal motion segment and the spinal organ, and will not be further addressed here. The significance of menisci in the lumbar zygapophyseal joints remains questionable. Bogduk [4, 5] described these menisci as rudimentary fibrous invaginations of the dorsal and ventral capsule. They are basically fat-filled synovial reflections, some of which contain dense fibrous tissue, which probably arises as a result of mechanical stress. Bogduk outlined that meniscal entrapment is probably an overstated cause of those forms of "acute locked back" that respond to manipulation [4].

Despite the recognition of the association between facet joint arthropathy and back pain by Goldthwait [6] in 1911, the facet joint was ignored until Mooney and Robertson [7] initiated a revival of the so-called "facet joint syndrome". Nevertheless, data on the pathogenesis of facet joint osteoarthritis are still very sparse in contrast to the knowledge gathered on synovial joint osteoarthritis [8–12].

As in large synovial joints, it has been assumed that mal-alignment is a predisposing factor for the development of osteoarthritis (OA). Fujiwara et al [13] examined the association between orientation and OA of the lumbar facet joints in 107 consecutive patients who underwent plain

radiography and magnetic resonance imaging. A significant association was found between sagittal orientation and osteoarthritis of the lumbar facet joints, even in patients without degenerative spondylolisthesis. The authors concluded that facet joint osteoarthritis, rather than spondylolisthesis, is the pathoanatomical feature that is associated with sagittal orientation of the facet joints in patients with degenerative spondylolisthesis.

Vernon-Roberts [14] outlined that, in contrast to osteoarthritis in large synovial joints (eg, hip joint), an intact covering of hyaline cartilage is frequently retained by the articular surfaces even when large osteophytes have formed. He hypothesized that this preservation of articular cartilage may result from changing joint stresses. However, Swanepoel et al [15] found that the apophyseal cartilage of the facet joint surfaces show a greater extent and prevalence of cartilage fibrillation than knee, hip, or ankle, with significant damage in specimens younger than 30 years. In late stages of OA, the facet joints also demonstrate the classic features, ie, complete loss of articular cartilage, cysts and pseudocysts in the bone, dense bone sclerosis, and large osteophyte formation. At this stage, end-plate fractures can occur which resemble breaches in the subchondral bone plate with protrusion of a portion of the articular cartilage into the subarticular bone [16]. Of importance is the notion that spontaneous fusion of the facet joints is very rare in the absence of ankylosing spondylitis or ankylosing hyperostosis [14].

Taylor and Twomey [17] investigated the degenerative changes of zygapophyseal joints in relation to biomechanical function. Their results indicated that articular cartilage and subchondral bone of the anterior, coronally oriented third of the joint show changes that are likely to be related to loading of this part of the joint in flexion. The posterior, sagittally oriented two-thirds of the joint show different age changes, which may reflect shearing forces imparted to the articular cartilage through the fibrous capsule, from insertion of some fibers of multifidus into the fibrous capsule. Swanepoel et al [15] explored the extent and location of fibrillated areas of the apophyseal cartilage of the joint surfaces of 29 lumbar motion segments. They found that damage was predominantly located peripherally, superiorly, and posteriorly in the concave superior apophyseal surfaces, and was predominantly peripheral and posterior in the inferior surfaces, with a tendency to be located inferiorly. The pattern of damage to the inferior surfaces lends some support to the hypothesis that their apices impact the laminae of the lumbar vertebra inferior to them, the result of degeneration and narrowing of the associated intervertebral disc. The predominantly peripheral location of fibrillation of both superior and inferior surfaces may be associated with inadequate mechanical conditioning of marginal joint areas [15]. In 24 (51%) of 47 facet joints analyzed histologically by Gries et al [18], the concave (or superior) facet was altered more severely than the convex (inferior) facet. In ten (21%) specimens, the convex facet showed more advanced changes. No difference was found in the remaining specimens. Gries et al [18] reported that the most severe changes were located in the posterior one-third of the joint in 16 (34%), in the anterior one-third in seven (15%), and equally distributed between anterior and posterior one-third in the remaining 14 (30%) specimens.

Fujiwara et al [19] investigated the effect of both disc degeneration and facet joint osteoarthritis on lumbar segmental motion. They reported that with cartilage degeneration of the facet joints, the axial rotational motion increased, whereas the lateral bending and flexion motion decreased in female segments. In male segments, however, motion in all directions increased with moderate cartilage damage and decreased

with severe cartilage degeneration. Subchondral sclerosis significantly decreased the motion, and the severity of osteophytes had no significant association with the segmental motion. However, the authors were not able to clarify whether facet joint OA differs between genders and how facet joint OA affects the stability of the spinal motion segment.

There is a void of information in the literature with regard to a comparison of the osteoarthritic changes in large weight-bearing synovial joints and facet joints on a histological and molecular level.

4 DEGENERATION OF THE MOTION SEGMENT

Based on the early work of Schmorl and Junghanns, Kirkaldy-Willis [20] proposed the concept of a close interaction between the two facet joints and the intervertebral disc in a so-called "three-joint complex" (**Fig 5.1-2**). Thus, degenerative alteration of the intervertebral disc will finally affect the facet joints and vice versa. According to the Kirkaldy-Willis' concept [20] progressive degenerative changes in the posterior joints lead to marked destruction and instability. Similar changes in the disc can result in herniation, internal disruption, and resorption. Combined changes in the posterior joint and disc sometimes produce entrapment of a spinal nerve in the lateral

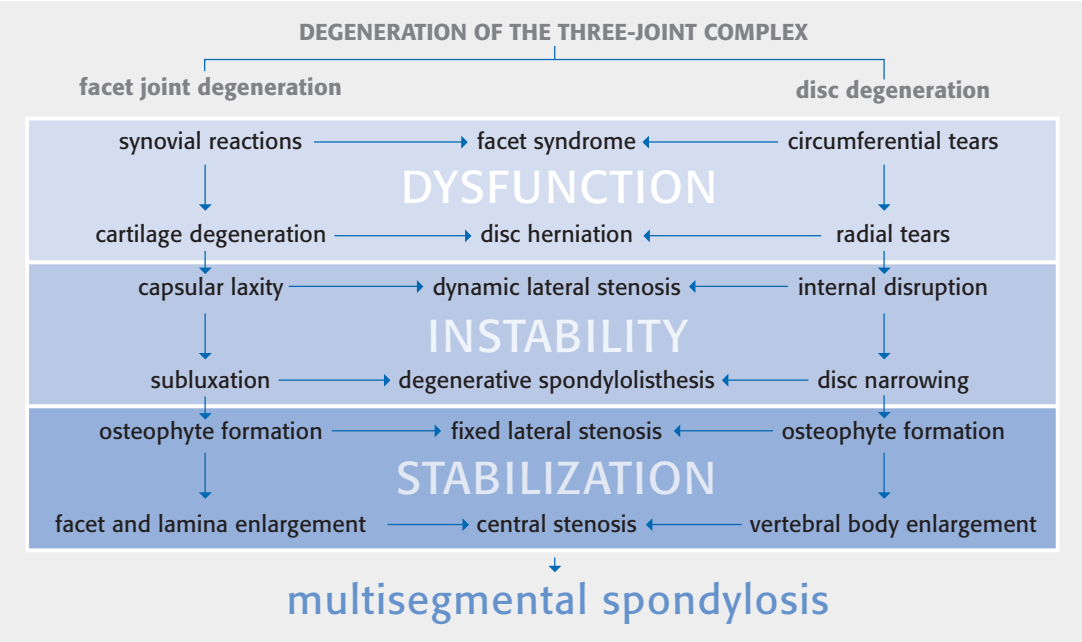

Fig 5.1-2
The degeneration of the so-called three-joint complex according to Kirkaldy-Willis [20] (modified).

recess, central stenosis at one level, or both of these conditions. Changes at one level often lead, over a period of years, to multilevel spondylosis and/or stenosis. Developmental stenosis is an enhancing factor in the presence of a small herniation or moderate degenerative stenosis (**Fig 5.1-2**).

Debate has continued on the temporal relationship between degenerative changes of the intervertebral disc and facet joints. Several studies have provided some evidence that disc degeneration usually precedes facet joint OA [21–24]. Although degeneration of the intervertebral disc results in decreased disc height, which alters the load distribution in the motion segment, the relation is not simple [3]. Gries et al [18] have shown that microscopic changes occur in discs and facet joints at an early age. However, the authors were not able to establish a close correlation with age, neither for the intervertebral disc nor for the facet joints at the same level. More interestingly, they reported that grades of disc degeneration did not correlate with those for the facet joints. From their results the authors concluded that it is not possible to demonstrate that pathological changes progress more rapidly in one element relative to the other in the young adult spine [18].

Notwithstanding its usefulness as a model for the study of the spine, Yeong-Hing and Kirkaldy-Willis [25] have outlined the limitations of the three-joint complex with regard to the effect of intrinsic and extrinsic action of the trunk muscles. At the level of the motion segment, there is only sparse data on the muscle function. Goel at al [26] have reported a three-dimensional, nonlinear, finite element model of a ligamentous L3/4 motion segment for the predictions of stresses in the motion segment. From their model the authors were able to show that the muscles imparted stability to the ligamentous motion segment. The presence of muscles also led to a decrease in stresses in the vertebral body, the intradiscal pressure, and other mechanical parameters of importance. However, the load bearing of the facets increased compared to the ligamentous model. Thus, facet joints play a significant role in transmitting loads in a normal intact spine. These results provide quantitative data on the stabilizing effects of muscles on the mechanics of a ligamentous spine. The results also provide a scientific explanation in support of the "degenerative cascade" concept proposed in the literature. The model predictions, in conjunction with the degenerative cascade concept, also support the observation that the osteoarthritis of facets may follow disc degeneration. There is a lack of studies exploring the concept of the so-called degenerative cascade in other regions of the spine, particularly the cervical region.

A conclusive and comprehensive assessment of the behavior of the muscles on the motion segment cannot be achieved with in vitro models alone, but requires in vivo models. In an animal model, Kaigle et al [27] studied the in vivo kinematics of a degenerated lumbar motion segment. From their results, the authors concluded that the lumbar paraspinal muscles are less efficient overall in providing stability during flexion-extension when chronic lesions are made in the intervertebral disc and facet joints. This is due possibly to altered mechanisms in the neuromuscular feedback system in the degenerated motion segment and, consequently, in the lumbar spine as a whole. These neuromuscular feedback mechanisms need to be incorporated in any model when we want to better understand the function of the motion segment in health and disease.

5 ALTERATIONS OF THE POSTERIOR SUPPORT STRUCTURES AND PAIN

The prerequisite for the transmission of pain is a neural innervation of the structure. Bogduk provided a comprehensive review of the neural innervation of the lumbar spine [4], highlighting the importance of the innervation of the posterior support structures, which are innervated by branches of the dorsal rami. The facet joint capsule is richly innervated with C and A delta fibers [28] and exhibit a neural innervation arising from the same level but also from the level above [29]. Cavanaugh [28] has outlined that under normal conditions pain fibers (nociceptors) have high mechanical thresholds. Under pathological conditions, such as inflammation, chemical mediators sensitize the nerve endings and they can begin to fire spontaneously, ie, with levels of physiological stress and strain. Neuromediators that contribute to the transmission process, particularly substance P, have been observed in the facet joint capsules [30, 31].

Based on the results of neurophysiological and neuroanatomical studies in anesthetized New Zealand white rabbits, Cavanaugh [28, 32] summarized the evidence in support of facet pain:

- An extensive distribution of small nerve fibers and endings in the lumbar facet joint.
- Nerves containing substance P.
- High threshold mechanoreceptors in the facet joint capsule.
- Sensitization and excitation of nerves in facet joint and surrounding muscle when the nerves were exposed to inflammatory or algesic chemicals.

An acute traumatic strain in the facet joint capsule could lead to inflammation and subsequently prolonged nociceptor excitation.

The incorporation of the mechanisms of pain generation, transmission, and modulation in the concept of back pain resulting from degenerative disorders of the motion segment is still at its beginning. Based on the results of imaging studies in asymptomatic individuals [33–35], degenerative changes may not per se be painful. A research approach focusing on pain generation and modulation is therefore more sensible and more likely to be successful in the vast majority of the patients where the ultimate clinical goal is to relieve pain.

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5 BIOLOGY OF THE SPINE
5.2 AGING AND PATHOLOGICAL DEGENERATION

5.2 AGING AND PATHOLOGICAL DEGENERATION

1 INTRODUCTION

The intervertebral discs separate the vertebrae of the spine where they facilitate the bending and twisting motion to which the spine is subjected and they also cushion compressive loading due to impact or gravity. These functions are endowed by the unique structure of the intervertebral disc, which is initially established during embryonic development and evolves throughout life. The vertebral column develops in the embryonic mesoderm at about 4 weeks gestational age in humans [1]. Vertebral bodies mature under the combined influence of the notochord and neuronal tube. Discs grow initially in an environment that contains few blood vessels and are surrounded by a perichondral layer, the future longitudinal vertebral ligaments. Between the vertebrae, the notochord expands as local aggregations of cells (the notochordal cells) within a proteoglycan matrix, forming the gelatinous center of the intervertebral disc, the nucleus pulposus. The nucleus is later surrounded by the circularly arranged fibrous lamellae of the anulus fibrosus, which are derived from the perichordal mesenchyme. The rapid increase in notochordal nucleus pulposus volume in fetuses occurs at the expense of the inner annular region. At this point, the cells populating the nucleus pulposus are a mixture of notochordal and chondrocyte-like cells, while the rest of the intervertebral disc contains fibroblast-like cells. At the junction with the notochordal sheath, the cells of the inner annulus are closer in shape to the chondrocyte-like cells found within the nucleus. The exact role and interaction of notochordal cells with the other disc cells is unknown.

The lumbar intervertebral disc undergoes very extensive destructive changes with age and degeneration [2]. The degree of this tissue destruction is closely linked to age, however, different components of the disc undergo more extensive alterations than others [3]. Although several investigations confirm the notion that disc degeneration is extensively seen in advanced age individuals, previous personal investigation has shown that initial degenerative alterations on the histological level are even visible in infantile discs [4]. Therefore,

substantial individual differences can be observed in the sense that young individuals exhibit the discs of an elderly person and vice versa. Because of the extensive destructive changes that ultimately lead to an ankylosed motion segment [4, 5], many clinicians and researchers believe that the intervertebral disc is a predominant source of low back pain. From a clinical point of view, differentiating "normal" age-related (ie, asymptomatic) from "pathological" degenerative (ie, painful) changes would be sensible. However, this task is extremely difficult due to the lack of a reference standard for painful disc degeneration. So far, the best reference standard is provocative discography. However, the role and potential benefit of this diagnostic procedure is controversially discussed in the literature [6].

For the purpose of this review, the focus will be on those morphological and molecular alterations which may be involved in the development of a painful degenerated disc. However, the authors do not imply that the alterations described within this chapter are exclusively found in individuals with a painful degenerated disc. Therefore, the term "degenerated" disc is not synonymous with painful disc.

2 MORPHOLOGY OF THE DEGENERATED INTERVERTEBRAL DISC

There exist several studies on the macro- and histomorphology of the intervertebral disc [3–5, 7] that will not be reviewed here in more detail. In summary, all these analyses indicate that there is a progressive destruction of the disc tissue (**Fig 5.2-1**), mainly starting in the nucleus and extending to the annulus, that is accompanied by reactive cellular proliferation (**Fig 5.2-2**) and granular and mucoid matrix degeneration (**Fig 5.2-3**, **Fig 5.2-4**). Finally, the regular disc structure is lost and frequently a replacement of the disc by a scar-like tissue is seen. These changes are associated with increasing age.

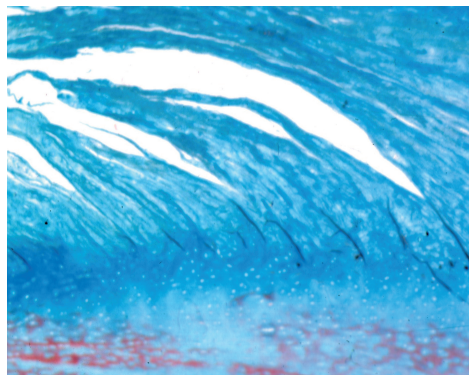

Fig 5.2-1
Histomorphological features of disc degeneration. Here, extensive formation of tissue clefts within the nucleus pulposus suggests major disruption of the matrix (22-year-old person, original magnification ×250).

3 INNERVATION OF INTERVERTEBRAL DISC

It is obvious that innervation of the disc may be crucial for the development of discogenic pain. In this regard, it is of significant interest that the disc is one of the largest aneural tissues of the human body. There is good evidence that discs are particularly free of nerve endings within their central portions [8, 9]. Only adjacent to capillary vessels in the outermost zone of the anulus fibrosus (AF) are small immunohistochemically detectable nerves identifiable [8].

As yet, we have no information whether these are only small nerves for vessel wall innervation or if they contain sensory components. There is still an open, undecided debate whether discal structures contain neurosecretory granules that may diffuse to juxta-discal receptors and thereby induce any signalling. This also holds true for previous studies [9, 10] that suggested the ingrowth of veritable nerves into degenerated discs.

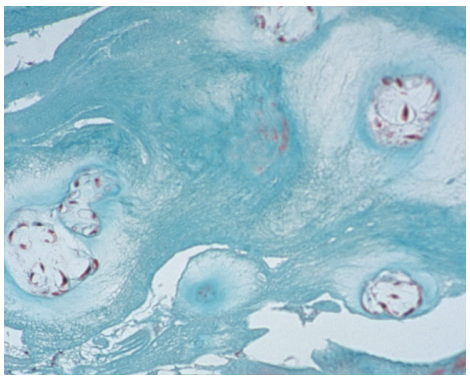

Fig 5.2-2
Histological signs of chondrocyte proliferation. Frequently, cluster-like proliferations of chondrocytes are present in disc tissue suggesting an attempt to restore the disc function (28-year-old person, original magnification × 400).

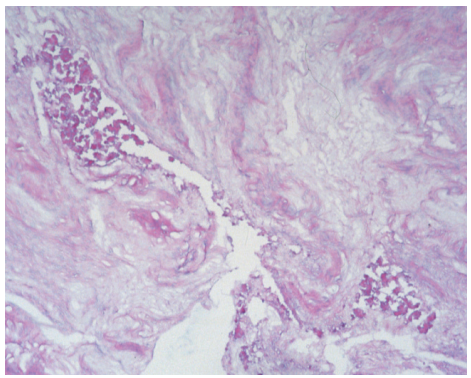

Fig 5.2-3
Histological signs for granular matrix degeneration with the local deposition of amorphous eosinophilic debris (21-year-old person, original magnification × 400).

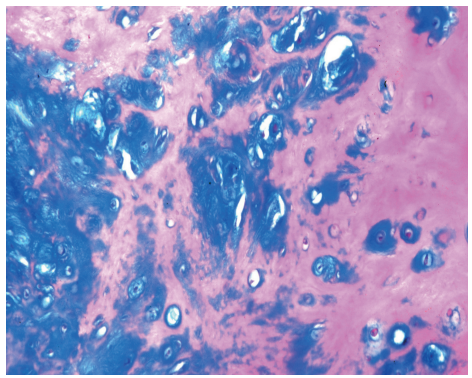

Fig 5.2-4
Histological features of mucoid matrix degeneration. This is characterized by the deposition of extensive amounts of mucoid matrix material (62-year-old person, original magnification × 250).

4 VASCULAR CHANGES DURING DISC DEGENERATION

Besides innervation the intervertebral discs are unique in that they lack any significant vascular supply [3, 4, 9, 11]. Accordingly, it has been assumed that the vascular supply of the intervertebral disc seems to be of most significance for the development of age-associated and degenerative alterations. While fetal and infantile discs contain vascular loops, the juvenile, adolescent, and adult disc is nonvascularized [12]. Therefore, the adult disc is the largest avascular tissue structure of the human body. Nutritional supply is provided predominantly by diffusion with a maximum diffusion distance of up to 1 cm (from the vertebral bone marrow to the center of the nucleus pulposus, NP). The only exception is the nutritional supply of the outermost zone of the AF, where small capillary blood vessels extend from the adjacent longitudinal ligaments between the lamellae of the AF [12] (**Fig 5.2-5**). All of the studies that have investigated the relative importance of these two sources of nutrition have supported the general consensus that the central region of the end plate is the predominant route of transport for metabolic processes of the disc [13–15]. For small solutes, it has been shown that diffusion is the predominant molecular transport mechanism from the end plate to the nucleus [16]. It has also been suggested that convective solute transport may play a role in nourishing the disc [17–19], but it is believed to be of importance only for the transport of larger solutes [16, 20]. Worthy of note, reduced permeability of end plates is generally seen in association with disc degeneration and age related changes [21]. Both of these changes may be due to calcification of the end plates and occlusion of the marrow contact channels observed with disease and age [22–24]. Hence, with decreased nutrient and metabolite transport, it is believed that the cells are unable to maintain the matrix necessary for the normal function of the disc, resulting in degeneration [24].

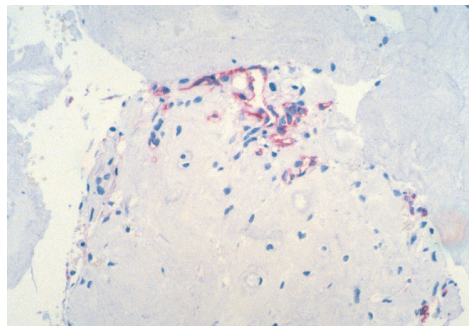

Fig 5.2-5
Immunolocalization of small blood vessels in the outer anulus fibrosus by staining of endothelial cells (CD 31). Normal discs show few small capillaries only in the outer zone of the anulus fibrosus (anti-CD 31, original magnification × 400).

In degenerated discs, the ingrowth of small capillary blood vessels is seen in those areas that present with a disruption of the matrix and the formation of clefts and tears. This, however, is only seen in annular lesions, but not in nuclear clefts. The presence of blood vessels is particularly seen in disc herniation (**Fig 5.2-6**).

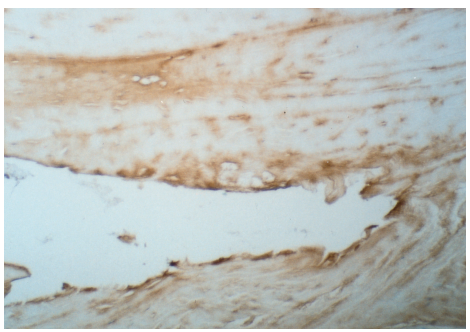

Fig 5.2-6
Increased formation of small blood vessels in herniated disc material. The immunolocalization of blood vessels in herniated disc tissue reveals the occurrence of small capillary buds close to clefts (anti-CD 31, original magnification × 250).

5 CHANGES OF THE EXTRACELLULAR MATRIX DURING DISC DEGENERATION

The structure and composition of the extracellular matrix are of fundamental significance for the biomechanical properties of the intervertebral disc.

5.1 COLLAGENS

The main structural component of the discal extracellular matrix is represented by collagen which is seen in the different anatomical subsettings with a variable composition of isoforms [11, 25, 26]. Several different collagen types have been identified in normal intervertebral discs (ie, types I, II, III, V, VI, IX, and XI) with some differences between the various anatomical regions. While the overall collagen content in the NP remains fairly constant over the years, that of the AF decreases with advancing age. In addition to these quantitative changes in the collagen content, major alterations occur with the tissue distribution of various collagen isoforms. This affects either those collagen types that are normally present within disc structures but which may reveal a quantitative or qualitative change, or may affect collagen types that are not normally present within the disc tissue.

The most obvious example for an "abnormally" occurring collagen molecule within aging (and degenerating) discs is the appearance of collagen type X in those discs of individuals of advanced age. This collagen molecule—"physiologically" expressed by hypertrophic chondrocytes during the growth period of long bones and therefore present in the notochord of fetal/infantile discs and in the growth zone of the end plate [27]—is completely absent in discs from individuals aged between 20 and 60 years, but reoccurs focally (and in very low amounts) associated with degenerative disc lesions of old-age individuals [28, 29]. Similarly, the authors observed the abortive expression of fragments of basement membrane

collagen type IV in discs from individuals between 20 and 50 years of age. This collagen molecule which normally is exclusively seen in epithelial, endothelial, or pericellular basement membranes seems to indicate partial changes in disc cell differentiation [11].

In addition, there is a significant change in the distribution of collagens with disc degeneration. Likewise, collagen type I is significantly expressed in NP tissue along with enhanced deposition of collagen types III and VI. In parallel, the content of collagen type II seems to be reduced in NP tissue, while the AF also contains more collagen III and VI in the aged and more degenerated situation. Similarly, EP tissue reveals the "abnormal" expression of collagen type III and VI which also parallels morphological signs for tissue disarrangement [11, 25].

5.2 PROTEOGLYCANS

With aging and degeneration comes a marked decrease in proteoglycan content in the nucleus (**Fig 5.2-7**) and significant alterations in proteoglycan structure [30–32]. This particularly affects aggrecan (**Fig 5.2-8**), which is the most abundant proteoglycan, and versican, another proteoglycan with the ability to form aggregates with hyaluronate [33]. As a consequence of these changes, the ability of the nucleus tissue to imbibe water and to radially and homogeneously distribute the compressive forces will progressively diminish with increased degeneration [34].

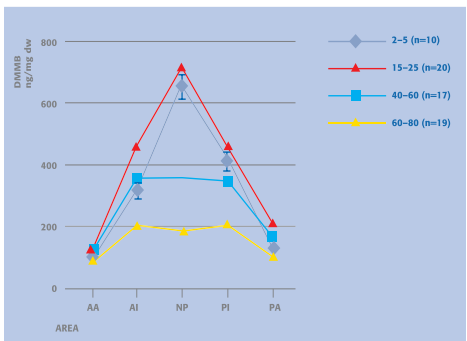

Fig 5.2-7
Changes in the proteoglycan amount, measured as glycosaminoglycan content, related to age within the different intervertebral disc regions.

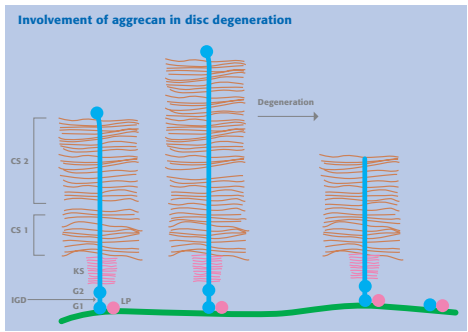

Fig 5.2-8
Aggrecan is represented as a core protein with three globular domains (G1, G2, and G3). The G1 and G2 domains are separated by an interglobular domain (IGD) and the G2 and G3 domains are separated by glycosaminoglycan-attachment domains bearing predominantly keratan sulfate chains (KS domain) or chondroitin sulfate chains (CS1 and CS2 domains). The CS1 domain possesses a variable number of tandem repeats (polymorphism), so that individuals may have aggrecan core proteins that can be short (left molecule) or long (middle left molecule). Disc degeneration involves proteolytic cleavage of the aggrecan core protein, often within the CS2 domain or IGD, resulting in fragments enriched in the CS1 domain (middle right molecule) or the G1 domain (right molecule). These fragments and the intact aggrecan are localized in the tissue via their interaction with hyaluronan (HA) and stabilized by link proteins (LP). (Courtesy of Dr Peter Roughley, Shriners Hospital for Children, Montreal, Canada.)

5.3 NONCOLLAGENOUS PROTEINS

Noncollagenous proteins represent up to 45% of the dry weight of the nucleus pulposus and 25% of the anulus fibrosus in human discs [35]. Several of the identifiable noncollagenous proteins include fibronectin, thrombospondin, and elastin. Preliminary personal analyses provide evidence that there exist age- and degeneration-related changes in at least some of these noncollagenous proteins of the disc. Thus, the authors have shown by immunohistochemistry and nonradioactive in situ hybridization that fibronectin is synthesized in enhanced amounts in those areas of morphological degeneration (**Fig 5.2-9**). Therefore, it can be assumed that these noncollagenous proteins may be involved in the age- and degeneration-associated matrix remodeling. The specific role of these proteins, however, remains to be elucidated.

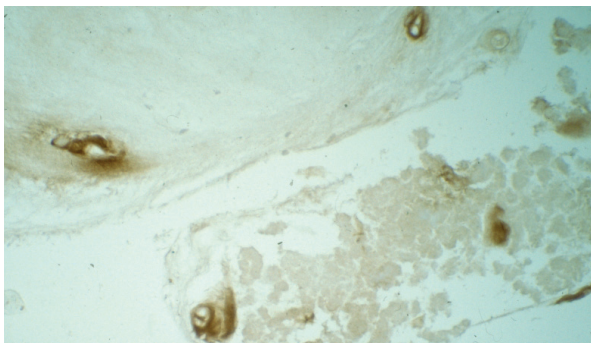

Fig 5.2-9
Immunolocalization of fibronectin in degenerated disc material showing a widespread and enhanced deposition of this matrix molecule (antifibronectin; original magnification × 100).

6 PROTEOLYTIC ACTIVITY IN DEGENERATED DISCS

A major hallmark of discs is the loss of height during disc degeneration [36, 37]. In addition, as already indicated above, the occurrence of clefts and tears is seen to a greater extent in areas of disc degeneration in adults [4]. These observations indicate that matrix molecules are degraded. A perturbation of the turnover of collagen molecules and proteoglycans in turn explains a loss of biomechanical stability and a weakening of the functional properties of affected discs. It is generally accepted that proteinases play a major role in this process [32, 38, 39].

The primary proteinases thought to be involved in the direct destruction of the disc tissue are the matrix metalloproteinases (MMPs) [32, 38, 39]. Within this family of proteolytic enzymes, there exist various groups that differ in their substrate specificity and thus in their proteolytic capacity. Intact interstitial collagen molecules, such as collagen types I, II, or III can only be degraded by the interstitial collagenases, with MMP-1 being the most important and widespread enzyme [32, 38, 39]. The other collagenases that can degrade intact interstitial collagen molecules are synthesized by polymorphonuclear leukocytes (MMP-8) or are not yet analyzed in disc material (eg, MMP-13). PMN-leukocytes are usually not present in disc material. Denatured collagen molecules can be further cleaved by the two gelatinases (MMP-2 and MMP-9) while the stromelysins (MMP-3, MMP-10, and MMP-11) degrade both denatured collagen and noncollagenous proteins, such as fibronectin and others. Proteoglycans are also cleaved by the stromelysins, including aggrecan and versican. Once activated, for example, by an enzymatic conversion of the proenzyme to the active enzyme, the MMPs cleave their substrate until they are inhibited by specific tissue-inhibitors-of-matrix-metalloproteinases (TIMPs) which exist in the human body with three isoforms. The balance between MMPs and TIMPs therefore control the level of proteolytic activity.

Under normal conditions, MMPs are synthesized in human lumbar disc tissue on a low, basal level [32, 39]. Previous extensive studies on the occurrence of MMPs (by immunohistochemistry) and their mRNA (by in situ hybridization) suggest that this synthesis is up-regulated in those areas with morphological signs of tissue degeneration, such as cleft formation (**Fig 5.2-10**). Besides the presence of enhanced amounts of various MMPs, the authors [39] and others [38] have shown by in situ zymography that enhanced foci of tissue proteolysis are indeed associated with cleft formation and disc tissue disruption (**Fig 5.2-11**). These observations clearly indicate that the up-regulation and activation of various MMPs is an important step in the degradation of the disc matrix finally leading to a disruption of the disc tissue. However, as yet no information is available on any changes of TIMP levels and it may be speculated that an enhanced synthesis of those TIMPs could represent a therapeutic option to prevent tissue disruption.

In addition to the MMPs, two further relevant enzymes have become a focus of disc tissue research. Thus, it has been described that the two aggrecanases (which are members of the ADAMTS-enzyme family) are also found in degenerated disc tissue [32, 38]. Both have similar substrate specificity and their activation may contribute significantly to the proteolysis of proteoglycans. The loss of proteoglycans in turn leads to a reduced water-binding capacity of the affected discs and thereby to a loss of function.

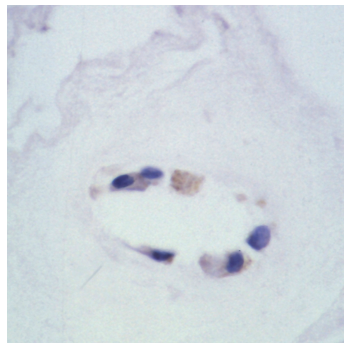

Fig 5.2-10
Immunohistochemical identification of matrix metalloproteinase MMP-1 positive cells in degenerated nuclear disc tissue. This collagenase is expressed in areas with evidence for tissue degeneration in enhanced amounts (anti-MMP-1; original magnification × 400).

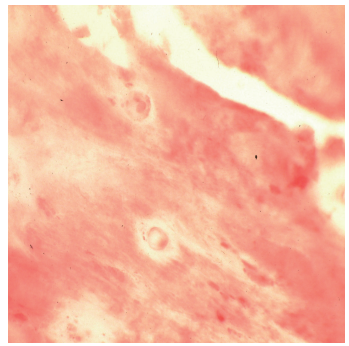

Fig 5.2-11
In situ localization of proteolytic activity by in situ zymography of degenerative altered NP material. The dissolution of an underlying gelatine matrix (light areas) suggests the presence and activation of matrix-degrading enzymes (in situ zymography, original magnification × 250).

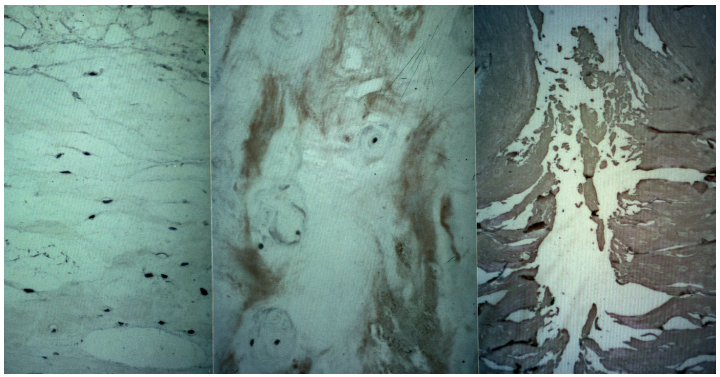

Fig 5.2-12
Immunolocalization of the oxidation end product carboxymethyllysin (CML) in degenerated nuclear disc material. This metabolic product is the result of accumulated oxidative stress and is normally not present in disc material (anti-CML, original magnification × 250).

7 EVIDENCE FOR ENHANCED OXIDATIVE STRESS IN DEGENERATING DISC TISSUE

Recent studies provide increasing evidence that oxidative waste products occur in disc tissue in association with degenerative alterations. This is even obvious by the accumulation of brown products in degenerated disc material [40]. These metabolic products may play a significant role in the aforementioned induction of synthesis and/or activation of cytokines and matrix metalloproteinases in discs.

Likewise, previous analyses revealed the deposition of a stable and irreversible end product of oxidative reactions, the specific amino acid modification carboxymethyllysin (CML) [41], to be increasingly present in discs with histological degenerative lesions [11] (**Fig 5.2-12**). The deposition of CML occurred as early as 16 years of age when initial significant histological signs of degeneration are seen. The CML-formation increased steadily in the nucleus pulposus from approximately the 20th to the 85th year of age and was also seen slightly later in annular disc areas and end-plate cartilage [11]. The occurrence of this marker modification indicative of oxidative stress strongly suggests that the intradiscal accumulation of oxidation products is an early and increasing reaction, obviously on the grounds of local hypoxia of disc cells, which in turn may trigger subsequent events of tissue destruction and disarrangement. Although the final proof for a direct role of CML as an inductor for cytokine production has not been accomplished, there is increasing evidence that the formation of CML-modifications of long-living matrix molecules enhances biosynthetic pathways of transmitter substances, matrix molecules and matrix-degrading enzymes [41]. The further investigation of the occurrence and formation of CML-modifications will provide potential insight into the initial metabolic changes that may induce or enhance disc degeneration.

8 PHENOTYPIC CHANGES OF DISC CELLS

Since the matrix is actively remodeled by the disc cells, the aforementioned changes in the composition of the extracellular matrix are induced, controlled, and executed by specific cells. In this regard, it is of interest that within the various anatomical settings significant differences in the phenotype can be assumed. However, the exact definition of the discal cellular phenotype is at present very difficult to establish.

Although all disc cells have previously been classified as chondrocytic cells—with the exception of fibroblastic cells of the outer AF—there seem to be major differences between those "chondrocytes" within various settings. Since there is no general marker that defines chondrocytes, a phenotypic classification is difficult and at present still insufficient. A generally accepted, but not specific marker molecule (which is also present on certain other cell types, such as some types of macrophages, adipocytes, melanocytes, and a few other cell types) is the S100-protein [42]. Using this molecule as a defining criterion of disc cells as "chondrocytes", almost all cells of the NP and EP and most of the cells of the inner AF can be classified as "chondrocytes" which is evidenced by positive cellular labelling.

Recently, Sive et al [43] used a panel of the three "marker molecules" collagen type II, aggrecan and Sox 9 to define chondrocytes in disc material. Using these markers and in situ hybridization techniques to identify mRNA-expression, the authors describe the presence of all three markers in normal NP material, but a reduction of aggrecan-mRNA expression in degenerated nuclear samples suggesting some changes in the cellular phenotype during degeneration.

When using the complete interstitial matrix composition as a definition criterion, the chondrocytes of the EP can be regarded as being closely related to articular chondrocytes, while those of the (inner) AF are ranked into the group of fibrochondrocytes, such as seen in discs or menisci of other body regions. The phenotype of the NP "chondrocytes" is somehow intermediate between the two.

There exists only indirect evidence for phenotypic changes of disc cells as evidenced from the above-described changes in the pericellular matrix. In addition, a recent personal analysis provides evidence that some disc "chondrocytes" undergo a specific phenotypic change during degeneration. This has been shown by the immunohistochemical analysis of the expression pattern of the CD-68 molecule, a lysosomal protein which is typically found in a series of cell types that all share phagocytic properties. In this study [44], the authors provided evidence that CD-68 positive phagocytic cells occur exclusively in disc tissue with morphological signs of disc degeneration and that those cells are seen mainly adjacent to areas with tissue disruption. These observations suggest that part of the disc cells undergo a phenotypic "switch" to phagocytic cells which may be the result of altered environmental conditions. A further phenotypic analysis of disc cells seems to be required in order to identify further cellular alterations during aging and disc degeneration.

Furthermore, recent studies [45, 46] suggest that at sites of herniation inflammatory cells locally invade the disc. These are mainly macrophages, but also mast cells that are assumed to liberate factors that may promote sensory irritation.

9 MODULATION OF DISC CELLS BY CYTOKINES AND GROWTH FACTORS

Until now more and more information has been gathered about the composition of the disc during aging and degeneration, and certain important alterations in the associated cellular and molecular features have been identified. However, only very little is known about the underlying modulating factors, their potential source and effects. This is mainly due to a technically problematic identification and quantitation of those factors within disc tissue material because the tissue concentrations may be very low and the turnover rate of those factors may be high. Therefore, only a few studies are available that use either isolated cells cultivated under in vitro conditions or tissue samples for (immuno-) morphological analysis [47–49].

In this system it can be assumed that a multitude of potential factors is available that may influence the growth pattern and biosynthetic capacity of disc cells. Out of this bulk of factors, some may be selected which may have a significant influence on disc metabolism. With regard to this, the authors previously suggested that TGF-ß may be of interest, since this cytokine plays an important role in controlling cell proliferation, MMP synthesis, and production of matrix molecules, and all three parameters seem to be disturbed during disc degeneration.

Although only limited information is available, in vitro studies revealed an enhanced responsiveness of disc cells to TGF-ß1 [50, 51] suggesting increased sensitivity of isolated disc cells to this factor. Recently, the authors detected enhanced amounts of TGF-ß1 producing cells within discs showing an increasing degree of morphological degeneration. This was confirmed by immunohistochemistry (for TGF-ß1 protein) and nonradioactive in situ hybridization (for TGF-ß1-mRNA). Thereby, the notion was corroborated that an enhanced TGF-ß-expression may contribute to increased matrix protein synthesis and thereby may promote matrix remodelling. Although this study clearly showed that TGF-ß1 is synthesized by local disc cells, we do not know the underlying stimulus for its production.

Furthermore, initial data are available about the temporospatial distribution of a further cytokine within the disc which the authors assume to play a major role in the enhancement of any "inflammatory" reaction [49]. Similarly, data has been accumulated that support such an "inflammatory" reaction. Therefore, TNF-α has been investigated immunomorphologically in various human lumbar disc samples and has also been shown to be increasingly expressed in association with disc degeneration. Since TNF-α is a potent proinflammatory cytokine, this enhanced expression may indicate an inflammatory reaction of local disc cells that may stimulate the liberation of further, pain-inducing neurotropic factors. Ultimately, the activation of the TNF-α pathway may lead to pain induction in the juxta-discal spaces that are well innervated. The confirmation of these data and the corroboration of this hypothesis seem to be of utmost importance for the understanding of discogenic pain induction.

10 A CURRENT CONCEPT OF PAINFUL DISC DEGENERATION

Taking all these observations and experimental information together, the authors feel that a current concept of degenerative disc pathogenesis and the generation of discogenic pain can be developed. Therefore, the following sequence of events may be crucial in inducing disc degeneration:

Beginning in early childhood, the closure of the blood vessels that enter the discs leads to a progressive "malnourishment" of disc cells [4]. This is significantly aggravated during the period of longitudinal growth of the body during puberty and it may also be worsened by structural disarrangements of the EP including calcifications, etc. These structural changes in the EP may lead to an occlusion of the marrow contact channels which in turn further diminishes nutritional supply. Finally, the distance for the supply of the disc cells by diffusion extends to such a length that nuclear disc cells suffer from severe and irreversible hypoxic damage leading to a progressive collapse of those cells. This may be reflected by the accumulation of oxidative waste products such as the CML-modification of long-living proteins [11].

In order to adapt to these novel conditions of impaired nutritional supply, part of those cells may change their phenotype to a phagocytic cell type which is able to produce enhanced amounts of matrix degrading MMPs [38, 44]. Then, the cleavage of collagen molecules leads to a disruption of the disc structure and the formation of major discal clefts and tears. Although these clefts and tears may enhance the influx of nutritional supply, the functional integrity of the disc structure is lost. The mechanical properties of the disc become insufficient so that finally the complete motion segment loses its function. This phenotypic change along with the synthesis of proinflammatory cytokines, eg, TNF-α, leads to the induction of an "inflammatory" reaction and it can be assumed that the extensive clefts and tears may promote the rapid spread of those cytokines to sensory nerve endings in the well-innervated regions surrounding the spinal canal. Thereby, significant pain may be induced leading to disabling of the affected individual. However, the high prevalence of asymptomatic disc alterations indicates that additional factors are required which ultimately turn an asymptomatic condition into painful disc degeneration.

Although several aspects of this concept are still speculative, the authors believe that additional studies on this issue may further support this hypothesis. Only the elucidation of those presumed pathomechanisms may provide a causal option for a therapeutic interference.

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5 BIOLOGY OF THE SPINE
5.3 BIOLOGY OF THE OSTEOPOROTIC SPINE

5.3 BIOLOGY OF THE OSTEOPOROTIC SPINE

1 EPIDEMIOLOGY OF OSTEOPOROSIS

The proportion of older people in the total population is increasing with rising life expectancy and therefore, the importance of osteoporosis, a disease that manifests itself with ageing, is also increasing [1]. In 1900 the life expectancy of a newborn girl was less than 50 years; by 1999 it was 83 years. The number of women over the age of 65 will double by the year 2040. Today, osteoporosis is the most common skeletal disorder. Osteoporosis is so prevalent that vertebral fractures occur in 30% of all postmenopausal women and 20% of men over 50 [2–5].

Osteoporosis has become an enormous sociomedical problem. Unfortunately, only about 20–30% of the patients are treated. Management of the osteoporosis problem will only be possible with a successful diagnosis of osteoporosis, for example, with quantitative methods before the disease becomes evident in conventional x-rays, and then starting an appropriate "prophylactic" therapy.

The main problem in future will be to identify patients at risk, ie, patients who start to develop osteoporosis after menopause. Women at risk today include: those with premature menopause, a diet that is deficient in calcium/vitamin D3, a lack of physical activity, a family history, and smokers.

In the EPOS (European prospective osteoporosis study), which used over 7,000 men and women, it was found that after 3.6 years subjects who had a lower bone density (T-score less than –2.5 SD) at the beginning of the study had osteoporotic fractures of the spine 1.4 times more often than men and women with normal bone density [6–8]. The study complies with the requirements of evidence-based medicine in all criteria. This means that patients with a fracture risk can certainly be identified and then provided with an effective treatment.

In 1998 the European Parliament passed the resolution that osteodensitometry must be covered by the national health services and made available to the population in order to identify women with an osteoporosis risk.

2 DEFINITION OF OSTEOPOROSIS

The human skeleton consists (in about equal parts) of basic substance and hydroxyapatite. In osteopenia and osteoporosis (in contrast to osteomalacia) this ratio is more or less preserved, but total bone mass is reduced.

The Consensus Conferences in Copenhagen 1990, Hong Kong 1993, and Amsterdam 1996, defined osteoporosis: "Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture. The magnitude of peak bone mass and the rate of duration of bone loss determine the likelihood of developing osteoporosis."

This definition contains three key elements of osteoporosis [9]:

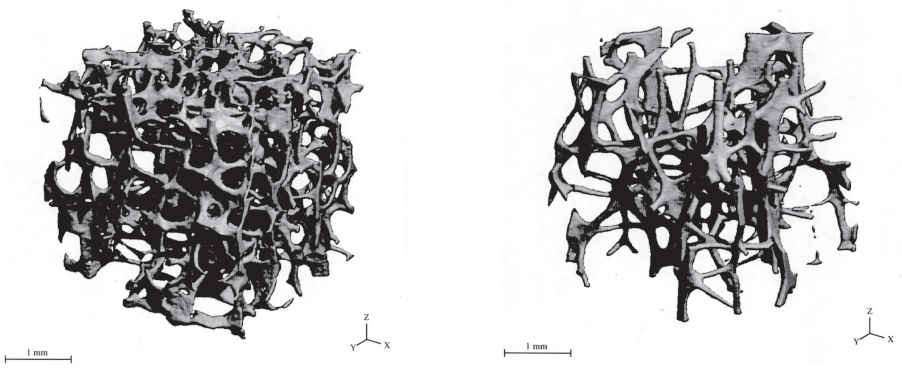
- Bone mass (how much is still left)
- Loss of bone mass (how much is lost)
- Microstructural changes (how is the bone structured)

In contrast to earlier years, the focus is now more on the **pathological** changes in structure, eg, how the trabeculae are linked, especially because they can now be made visible and measured not only in vitro (**Fig 5.3-1a–b**), but also in vivo.

In addition, the WHO quantifies osteoporosis based on a densitometric bone density assessment (**Fig 5.3-2**):

- Normal: T-score + or –1 SD
- Osteopenia: T-score –1 to –2.5 SD
- Osteoporosis: T-score – 2.5 or less

The authors define a state without fractures as **preclinical osteoporosis** and a state with fractures as **manifest osteoporosis** (–2.5 SD or less). In general, osteoporosis develops in episodes. In postmenopause, a high bone turnover (increased formation and resorption) is identical with rapid bone loss and is referred to as "fast-bone-loser". Although rapid trabecular bone loss occurs with an annual rate of more than 3.5%, after



a b

Fig 5.3-1a–b
Normal (a) and osteoporotic (b) bone structures (cancellous bone, vertebra) (µCT 20, Scanco Medical AG, Zürich).

the onset of menopause (in terms of the total group). Only 34% of women are at risk to develop osteoporosis. Vice versa, stability in severe age-related osteoporosis (formerly referred to as **senile osteoporosis**) has proved erroneous. In this form of osteoporosis, a fast-loser state is found in about 75% of the patients.

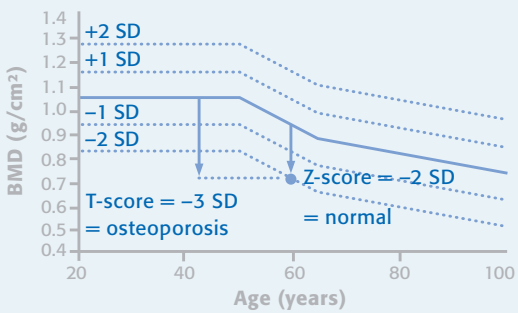


Fig 5.3-2
Definition of T-score and Z-score in osteodensitometry.
T-score expresses the deviation of a measurement from the mean value of healthy women aged 20–45 (peak bone mass) in mean ± SD.
Z-score expresses the deviation of a measurement from the mean average bone density of a peer population in standard deviation (± SD). This Z-score is hardly used any more today.

3 ETIOLOGY

3.1 PRIMARY OSTEOPOROSIS

The etiology of primary osteoporosis is still not completely understood. The most frequent form is postmenopausal osteoporosis (90%). Pathogenetic factors for the development of osteoporosis are:

- Estrogen deficiency
- Reduced calcium resorption
- Vitamin D deficiency

In contrast to women, the cause of osteoporosis in men is identified substantially less frequently with lack of testosterone being the most common cause.

3.2 SECONDARY OSTEOPOROSIS

In contrast to primary osteoporosis, secondary osteoporosis (**Table 5.3-1**) is secondary to a preexisting disease (eg, hypogonadism in women, anorexia nervosa, osteoporosis in professional dancers and athletes, or drug treatment) [10].

Corticosteroid osteoporosis

The most important secondary osteoporosis is caused by steroid treatment and is similar to the osteroporosis caused by Cushing syndrome. The likelihood of developing osteoporosis due to steroid treatment is related to the dose and the time period of use. However, there exits a substantial individual variation; not every patient treated with steroids develops osteoporosis. Some evidence shows that steroid treatment in doses less than 7.5 mg per day over 1 year is not a risk factor [11].

Synopsis of secondary osteoporosis

Inflammatory disease	Rheumatoid arthritis Inflammatory bowel disease Cystic fibrosis
Bone marrow disorders	Multiple myeloma Mastocytosis Leukemia
Disorders associated with immobilization	Parkinson disease Poliomyelitis Cerebral palsy Paraplegia
Defective synthesis of connective tissue	Osteogenesis imperfecta Marfan syndrome Homocystinuria
Disorders associated with hypogonadism	Athletic amenorrhea (marathon runner osteoporosis) Hemochromatosis Turner syndrome Klinefelter syndrome Postchemotherapy Hypopituitarism
Disorders associated with low body weight	Anorexia nervosa Diabetes mellitus type I
Disorders associated with malabsorption	Coeliac disease Postgastrectomy Liver disease Total parenteral nutrition
Endocrinological disorders	Thyreotoxicosis Hyperparathyroidism
Miscellaneous	Pregnancy/lactation Ankylosing spondylitis Hypercalciuric nephrolithiasis
Drugs	Alcohol Caffeine Anticonvulsants Methotrexate Heparin Cyclosporin

Table 5.3-1
Secondary osteoporosis as a consequence of a preexisting disease.

Hypogonadism in women

Apart from postmenopausal osteoporosis, accelerated bone loss may also occur in women after ovarectomy with estrogen deficiency. Remember that an estrogen deficiency may be present after a hysterectomy, even if the ovaries are left surgically intact, depending on the surgical technique (iatrogenic compromise of ovarian blood supply). Therefore, standard practice should determine the estrogen and gonadotropin levels when clinical signs of hypogonadism are present, even if the patient denies that the ovaries were removed at the hysterectomy.

Turner syndrome

This genetic anomaly causes a congenital form of hypogonadism in women (gonadal dysgenesis). These patients have normal female genitals, but rudimentary gonads without any function. Radiologically a coarse bone dystrophy with kyphosis and hypostosis can be found. If the syndrome is diagnosed late, eg, in adulthood, the authors frequently find that estrogen replacement, which would be the most obvious treatment, is not recommended in order to avoid undesirable psychological and physical perturbations.

Anorexia nervosa

Women with sustained anorexia nervosa frequently present a very severe, predominantly cancellous osteoporosis. The treatment of these seriously underweight patients with special nutrition often results in a further marked loss of cancellous bone, the cortical bone being less involved. In this context, it should be emphasized that esophageal/gastrointestinal symptoms are relevant side effects of bisphosphonates in daily practice, which in turn could lead to further decreased food intake.

Marathon runner osteoporosis

This functional disorder of the ovaries that causes hyperprolactinemic amenorrhea is commonly found in dancers and top athletes. Estrogen replacement therapy is necessary if such an amenorrhea lasts more than 6 months. Surprisingly, this problem is refuted or played down to an extent. This form of secondary osteoporosis is closely associated with anorexia nervosa and has similar psychological behavior patterns, including physical hyperactivity (best described as “being driven”).

Corticosteroid osteoporosis, osteoporosis associated with anorexia nervosa, and marathon runners osteoporosis, are characterized by a loss of cancellous bone rather than a loss of cortical bone.

4 DIAGNOSTIC ASSESSMENT

4.1 IMAGING STUDIES

Standard x-rays

Decreased bone density results in enhanced radiation permeability and cannot be detected radiologically until the loss of substance reaches a level of about 30–50%. Thereby, the patients' physical health (eg, obesity) and the radiological technique also play a role. In addition, the assessment of a reduced radiological shadow density may vary considerably. Therefore, the criterion "reduced radiological shadow density" should no longer be used. The diagnosis of osteoporosis using standard x-rays has thus been surpassed by modern densitometry methods, which are considerably more sensitive and reliable. However, standard x-rays still play a major role in the assessment of spinal deformity and of fractures.

Densitometry

Two methods are widely used, ie, dual x-ray absorptiometry (DXA) [10, 12] and high-resolution quantitative computed tomography hrpQCT [13]. A comparison of these two techniques, namely DXA and hrpQCT in multi-layer technique, is presented in **Table 5.3-2**. This demonstrates the great differences between the individual methods, both in terms of reproducibility, exposure, and location of measurement. The thin and multi-layer technique hrpQCT is the most sensitive. hrpQCT allows cancellous and cortical bone to be measured either together or separately at peripheral sites (radius and tibia) with minimum radiation exposure and with a low, thus optimal reproducibility. This is important because cancellous and cortical bone represent two different systems that may change in different ways and rates, both with regard to the development of osteoporosis and the therapy. For example, in steroid osteoporosis and osteoporosis associated with anorexia nervosa mainly the cancellous and less of the cortical bone is affected, while in primary hyperparathyroidism cortical bone is mainly affected. In hyperprolactinemic amenorrhea in young athletes ("marathon runner osteoporosis") a total loss of cancellous bone may occur. The rate of cancellous bone loss in menopause is about 1% per year in healthy women, 1–3.5% in "slow-loser" patients and more than 3.5% in "fast-loser" patients. Thus, quantitative densitometry methods must

	DXA	hrpQCT multi-layer/ thin-layer
Measurement sites	Lumbar spine, proximal femur, radius	Radius, tibia, hand
Parameters	Integral cortical with cancellous bone	Elective cancellous and cortical bone structure parameters
Dimension	g/cm² (surface value)	mg/m³ (volume value)
Reproducibility	± 1–2% (young healthy subjects)	± 0.3% (mixed collective)
Accuracy	3–6%	< 1%
Exposure (mSv)	< 0.05	< 0.1
Time/site (min)	approximately 10 min	4 slices/8 min 16 slices/16 min

Table 5.3-2
Comparison between the dual x-ray absorptiometry (DXA) and high-resolution peripheral quantitative computed tomography (hrpQCT) using thin- and multi-layer technique.

have a very good reproducibility to measure these differences and provide useful information for therapy decisions. The cancellous bone measured at the distal radius correlates well with the cancellous bone of the lumbar spine. The indications for densitometry are summarized in **Table 5.3-3**.

Of particular interest is the reproducibility of the measurements to assess the progression of the disease (eg, slow vs fast loser) and the treatment effect (eg, change in medication). The long-term reproducibility determines the minimum assessment intervals. The reproducibility data provided by the manufacturer are normally attained by a highly qualified investigator in healthy subjects, at short intervals, and under laboratory conditions; this is why it often deviates considerably from the long-term reproducibility in clinical practice.

The importance of reproducibility is illustrated by the following example: A patient with severe osteoporosis (eg, who has already lost more than 50% of bone mass) is examined using an osteodensitometry method with a long-term reproducibility in healthy subjects of ±2% (eg, DXA). In this case, the question arises as to which time interval should be chosen, eg, if a minimum change of ±3% per year is to be detected with 95% times certainty (**Table 5.3-4**). One needs to wait 45 months before a change of ±3% can be detected with certainty (95% confidence). In a clinical setting this is unacceptable for obvious reasons. If a method has a reproducibility of ±0.3% (eg, hrpQCT) than one only has to wait 7 months under the same conditions as above, thereby facilitating therapeutic decision making (**Table 5.3-4**).

Indications for densitometry
Confirmed indications
Manifest osteoporosis with fracture
Long-term glucocorticoid treatment
Hypogonadism
Anorexia
Chronic gastrointestinal disorders (eg, Crohn disease, malabsorption)
Primary hyperparathyroidism (unclear surgical indication, bone involvement)
Organ transplant (especially heart, lung, liver)
Osteogenesis imperfecta
Evaluation of therapy success
Identification of slow-loser and fast-loser patients
Possible indications
Family history of osteoporotic fractures
Estrogen deficiency syndrome
Menopause before the age of 45
Primary and secondary amenorrhea
Fractures after inadequate trauma
Radiological signs of osteoporosis (conventional x-ray)

Table 5.3-3
These indications vary from country to country, depending in particular on the health authorities and national health services.

Relative bone density	Reproducibility										
	±0.3%	±0.5%	±1.0%	±1.5%	±2.0%	±2.5%	±3.0%	±3.5%	±4.0%	±4.5%	±5.0%
120	3	5	9	14	19	24	28	33	38	42	47
110	3	5	10	15	21	26	31	36	41	46	51
100	3	6	11	17	23	28	34	40	45	51	57
90	4	6	13	19	25	31	38	44	50	57	63
80	4	7	14	21	28	35	42	50	57	64	71
70	5	8	16	24	32	40	49	57	65	73	81
60	6	9	19	28	38	47	57	66	75	85	94
50	7	11	23	34	45	57	68	79	91	102	113
40	8	14	28	42	57	71	85	99	113	127	142

Table 5.3-4
Minimum time intervals (in months) depending on bone density and reproducibility for identifying bone loss with a magnitude of ±3% on the 95% confidence level.

Bone scan

A bone scan is indicated in cases where a generalized bone disease, tumor, or infection is suspected. Although this method has a high sensitivity, its specificity is low. On the other hand, about twice as many metastases can be identified with scanning as with x-ray. The major advantage of a bone scan is that the relevant parts of the body (such as the spine, pelvis, skull, ribs, and the proximal tibia) can be imaged in a single examination [14].

Magnetic resonance imaging (MRI)

MRI is more sensitive than bone scans in the assessment of metastasis. A further advantage is the sensitivity to scintigraphically negative lesions, such as multiple myeloma, and the more precise anatomical demonstration of any infective or tumorous alterations [15, 16]. A fluid sensitive sequence (eg, STIR) can be helpful in deciding whether a vertebral compression fracture is acute, subacute, or already healed.

4.2 LABORATORY WORKUP

The laboratory work primarily aims to rule out secondary osteoporosis and is summarized in **Table 5.3-5**. A further detailed differentiation of the bone metabolism can be investigated using metabolic products and enzymes formed by the bone cells, and products of the bone matrix released into the serum, mainly during bone resorption [17].

The following **parameters for bone formation** are available in clinical practice:

Alkaline phosphatase

Alkaline phosphatase is not only found in bone, but also in the liver, kidneys, intestine, and placenta (alkaline phosphatase, isoenzymes). The amino acid sequence is identical, except differences in the tertiary structure. Alkaline phosphatase of the bone is localized in the membranes of the osteoblasts and it also plays a role in the mineralization of the osteoid. The enzyme has no circadian rhythm and is relatively stable after drawing blood. Raised serum levels are found in the presence of a increased bone turnover rate or mineralization disorder. In osteoporosis the values are usually within the normal range or slightly raised.

Osteocalcin

Synthesis of osteocalcin is controlled by calcitriol. Osteocalcin is 10–20% of the noncollagen proteins in the matrix. The precise function is still unknown. This probably plays a role in the mineralization of the osteoid. Osteocalin is integrated in the bone matrix, and about 20–30% is released into the serum. It can be quantified with specific immunoassays. While the half-life of alkaline phosphatase is 1–2 days, the half-life of osteocalcin is 4 minutes. Osteocalcin has a circadian rhythm with a maximum in the early hours of the morning. Because of rapid degradation, the samples must be processed very quickly. Increased levels are found in renal failure and during treatment with calcitriol.

Procollagen/propeptide

As mentioned above, the organic matrix consists of about 90% collagen type I. During integration in the bone matrix, amino-terminal, and carboxy-terminal fragments are separated from the procollagen type I molecule and secreted into the serum.

Level 1	Level 2	Level 3
Exclusion of secondary osteoporosis	Clinical suspicion of secondary osteoporosis	Dynamics of bone metabolism
Ca, P, alkaline phosphatase, osteocalcin, creatinine, bilirubin, SGOT/SGPT, erythrocyte sedimentation rate, serum immuno-electrophoresis, blood count, urine status	25(OH)D3 (malabsorption), parathyroid hormone, T4, TSH, testosterone, 1,25(OH)2D3 (renal osteodystrophy)	Osteocalcin (bone formation parameter), desoxypyridinoline/creatinine ratio (bone resorption parameter)

Table 5.3-5
Laboratory workup in patients with suspected osteoporosis.

The carboxy- and amino-terminal fragments can be measured in the serum using immunoassays. Thus, they represent the osteoblast collagen synthesis. There is a circadian rhythm, but the stability after taking the sample is greater than that of osteocalcin. The clinical value has not yet been fully explored.

The following **parameters for bone resorption** are available in clinical practice:

Hydroxyproline

Hydroxyproline is no longer used as a marker for bone resorption, since it requires a 3-day proline-free diet for measurement and the collection of 24-hour urine.

Pyridinoline cross-links

Pyridinoline and desoxypyridinoline are bone-specific and exhibit a circadian rhythm with the highest levels found in the early morning and the lowest in the afternoon. These substances are released during bone resorption and eliminated as free amino acids or as telopeptides. A specific diet prior to the urine sampling period is not required. The analysis method in urine is very complex. It may be expected that the pyridinoline cross-links in the serum will be determined more often in future. The advantage of this cross-link determination method in the serum is that a single blood sample could also be used to measure osteocalcin as a formation parameter, and cross-links as a resorption parameter.

Tartrate-resistant acid phosphatase

This enzyme is released in the osteoclasts, the prostate, and the hematopoietic system. Tartrate-resistant acid phosphatase is very unstable and samples must be processed immediately.

4.3 BONE BIOPSY

Since bone biopsy requires a surgical intervention and the processing of the biopsy is very complex, especially where the diagnosis of the activity of osteoporosis is concerned, this comes quite late in the diagnostic workup [18]. Recently, fewer bone biopsies have been performed because of the diagnostic accuracy of CT, MRI, and FDG-PET.

A bone biopsy is indicated:

- If a bone scan or MRI indicates "malignant" growth.
- If a hematological disorder is suspected.
- If the previous tests did not allow a clear distinction between osteoporosis and osteomalacia.
- In all cases of "unusual" osteoporosis, eg, in young women who are still menstruating or in young men.

The prerequisite for a morphometric evaluation of bone biopsies is, however, that the removed biopsy specimen is large enough and has not been destroyed for the evaluation of bone structures. When processing the samples in order that the tetracycline marker for identifying the mineralization front remains visible, they must not be decalcified. Only preparations that have not been decalcified will allow one to distinguish whether osteoidosis or true osteomalacia is present (tetracycline marker present or diffuse). Osteoidosis is found, for example, with high bone turnover (fluoride therapy), osteomalacia with malabsorption and maldigestion. The tetracycline marker is imperative for a correct interpretation of the bone biopsy. Preparations that have not been decalcified allow the calculation of morphometric structure parameters (if microCT/microtomography is not available), and in par-

ticular the quantitative measurement of the osteoblasts and osteoclasts. These parameters can then be used later for a specific therapy and show whether the bone loss shown objectively by quantitative computed tomography is due to osteoblast insufficiency or an increase in osteoclasts.

Drugs influencing bone mass
Substances that stimulate bone formation
Fluorides
Anabolics
Estrogens at high doses (eg, implants)
D-hormone preparations
PTH injections
Substances that inhibit bone resorption
Estrogens
Calcitonin
Bisphosphonates
Anabolics (anticatabolics)
D-hormone preparations
Calcium/vitamin D

Table 5.3-6

5 TREATMENT

Guidelines for the treatment of osteoporosis are far beyond the scope of this chapter; therefore, the authors only briefly review some general principles (**Table 5.3-6**). Obviously treatment of osteoporosis with bone stimulating and antiresorptive substances must be differentiated. The osteoporosis of each patient must be examined individually and therapeutic measures should be based on the dynamics of the disease [19]. In general, osteoblasts can be stimulated with fluorides and PTH (parathormone) given subcutaneously, while osteoclasts can be inhibited with estrogens, calcitonin, bisphosphonates, and D-hormone metabolites. An appropriate "prophylactic" therapy includes estrogens, SERMs (selective estrogen receptor modulators), estrogen-like substances, bisphosphonates, and calcium/vitamin D. In osteoporosis, treatment is dependent on the bone metabolism. Accordingly, in slow-loser patients drugs that promote formation and in fast-loser patients that inhibit resorption are predominantly used (**Table 5.3-6**).

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5 BIOLOGY OF THE SPINE
5.4 BIOLOGY OF FUSION WITH BONE AND BONE SUBSTITUTES

5 BIOLOGY OF THE SPINE

5.4 BIOLOGY OF FUSION WITH BONE AND BONE SUBSTITUTES

1 INTRODUCTION

During the last two decades the number of spinal surgery procedures has been steadily increasing and vertebral fusions even more so. In North America alone over 200,000 spinal arthrodeses are presently performed each year. Globally, the number is expected to show continued growth. Despite modern technological progress, the rate of failure to achieve a solid bony union (ie, pseudarthrosis or nonunion) is reported to vary between 5% and 35% with single-level fusions and to increase even more when multilevel arthrodeses are attempted [1].

The outcome of spinal fusion depends on a complex process influenced primarily by the type of graft material used and many local and systemic factors that affect the healing response of an arthrodesis (**Table 5.4-1**) [1]. The biomechanical aspect of the specific type of fusion (ie, posterolateral intertransverse process, anterior interbody, anteroposterior combined), the level of the arthrodesis, and the efficacy of spinal immobilization (internal or external) after surgery

Major factors affecting bone graft healing

Bone graft material	Type Quantity Sterilization technique
Local factors—biological	Preparation of recipient site Vascular supply of soft tissue Local bone disease (tumor, marrow infiltration disease) Radiation
Local factors—mechanical	Biomechanical stability Biomechanical loading
Systemic factors	Nutrition Hormones, growth factors Drugs Osteoporosis Infections Smoking

Table 5.4-1

will require special attention. Considerable progress in the technology of spinal instrumentation has almost maximized the benefits of mechanical stabilization, but the limiting step to arthrodesis remains the biological ability to form osseous consolidation between adjacent vertebral segments.

Recent advances in minimally or less invasive surgical techniques and the potential for biological regulation or manipulation of bone formation make it important to re-examine our understanding of the biology of bone graft materials and the spinal fusion process. This chapter will not review the multiplicity of local and systemic factors affecting spinal arthrodesis, but it will address the clinical applications of mineralized and demineralized bone graft preparations in spine surgery, reviewing the basic science and clinical experience supporting the use of these substitutes.

2 BIOLOGY OF SPINAL FUSION

A bone graft material is any implanted material that alone or in combination with other materials promotes a bone healing response by osteogenic, osteoconductive, or osteoinductive activity at a local site (**Fig 5.4-1**):

- Graft material that is **osteogenic** contains viable cells at some stage of osteoblastic differentiation that are, or potentially can be, capable of directly forming bone.
- **Osteoconductive** materials provide a biocompatible physical structure or scaffold that supports new bone formation [2, 3].
- **Osteoinductive** graft material contains cytokines capable of inducing differentiation of our undetermined osteoprogenitor stem cell to an osteogenic cell type.

The ideal bone graft material possesses three distinct properties—osteogenesis, osteoconduction, osteoinduction—with optimal immunological response in the host and without risk of disease transmission.

The **osteogenic potential** of a graft material is directly derived from its cellular content. It must contain viable cells that can form bone (osteogenic precursor cells). These cells participate in the early stages of the healing process to unite the graft to the host bone and their viability must be ensured during the grafting procedure. Fresh autogenous bone and bone marrow are the best known graft materials.

Osteoconductivity is the physical property of a graft material that allows the ingrowth of neovascularization and infiltration of osteogenic precursor cells. Appropriate osteoconduction is provided by direct apposition between host bone and implant. Host bone must be viable, the host-bone-implant interface

must be stabilized (no macromotion) and the implant needs a structure (porosity) allowing new bone ingrowth. Optimal interconnection between pores (connective porosity) and a pore size superior to 100 µm have been demonstrated by biological studies to be essential in this process.

Osteoinduction is the process by which some factors or substances stimulate the undetermined osteoprogenitor stem cell (responding cells) to induce the osteoblastic pathway and to differentiate into an osteogenic cell type (osteocyte). Osteoinductive properties have been found in demineralized bone matrix (DBM) and different morphogenetic proteins (BMP, TGF-β, PDGF, EGF, etc) (**Fig 5.4-1**).

2.1 AUTOGENOUS BONE GRAFT

Autogenous bone contains all of these three properties which promote bone fusion and it is considered as the gold standard among graft materials against which all others are compared. Corticocancellous bone, usually harvested from the iliac crest, has been the most common and most successful graft material in spinal fusion surgery. It is thought to contain both determined and inducible osteogenic precursor cells (osteogenic property), noncollagenous bone matrix proteins including growth factors (osteoinductive property), and bone mineral and collagen (osteoconductive property). Recent studies have also shown that all osteotropic growth factors known to be

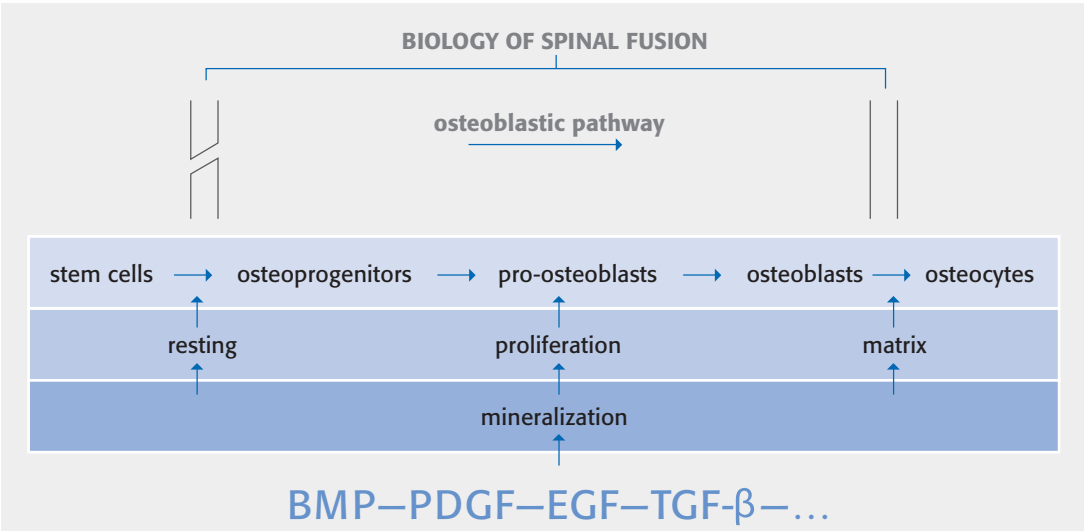

Fig 5.4-1

sequentially involved in the spinal fusion process are present in iliac crest bone autograft of all age groups but with a higher variation in older people (osteoinductive property) [4]. In addition, autogenous bone has the advantage of being non-immunogenic and nonpathogenic.

Cancellous bone contains greater osteogenic potential because of the large number of surviving cells in the marrow and because it is favorable to vascular ingrowth and exposure of inductive proteins due to the large trabecular surface area and interconnected spaces. Cortical bone offers greater mechanical strength compared to cancellous bone, but is less effective for the following reasons. There is less or no marrow, and consequently fewer osteogenic cells; its structure is less favorable for new bone formation and is more resistant to vascular ingrowth and remodeling [3].

Osseous spinal fusion remains a cornerstone of surgical treatment for severe spinal disorders. However, the success rate is still debated and difficult to assess in the presence of metal implant material. A general failure rate of autogenous bone graft arthrodesis has been reported as higher than 30% in some series and, although progress in spinal instrumentation has decreased this sequela, the incidence of nonunion has remained unacceptably high [1]. Moreover, the morbidity related to harvesting bone graft from the iliac crest for lumbar spinal fusion can sometimes be more problematic than the primary surgical procedure itself. Major complications such as pelvic fractures, vascular injuries, and deep infection have been reported in as many as 9% of patients, while minor complications including chronic donor site pain and superficial infection have been observed in up to 25% of the cases. The most common minor complication is the alteration in sensation over the donor site area, manifested as chronic pain, hyperesthesia, dysesthesia, or diminished sensitivity in the cutaneous nerve territory. The quantity of bone available from the iliac crest, the increased operative time and its related cost, the blood loss, and the possible need for transfusion are additional problems to be considered.

For these reasons, more and more attention has been directed in recent years to the development and use of bone graft substitutes or extenders. In spine surgery, the ideal bone graft substitute should be osteogenic, biocompatible, bioabsorbable, easy to use, and cost effective, it should also provide structural support. However, success in achieving these goals depends upon the material and its biological properties, as well as the particular host environment into which it is placed.

2.2 ALLOGRAFT

Historically, allograft has been the most common substitute for autogenous bone. It is highly osteoconductive, weakly osteoinductive, and not osteogenic, because cells do not survive transplantation. For these reasons, there has been concern as to whether allograft can reliably produce spinal fusion. Allografts may be available in a reasonable quantity and are quite versatile, in that the shape, contour, and mineral density of the graft can be defined by the specific part of the skeleton used to obtain the material and the machining that is performed.

Major concern exists among clinicians as well as the public about the possibility of infectious disease transmission, despite meticulous screening and serological testing of the donor [5]. Allografts are processed and preserved in ways that affect the osteoinductive and osteoconductive capacity of the material as

well as its immunogenicity. Preservation is obtained by either fresh-freezing or freeze-drying, both of which allow extended storage but reduce immunogenicity of the graft and may alter its mechanical strength and leave the worrisome risk of viral disease transmission. Further sterilization with high-dose gamma irradiation or ethylene oxide gas is used, but both methods may further reduce osteoinductivity. Ethylene oxide sterilization is believed by most clinicians to prevent viral infection. However, studies have shown that the gas fails to penetrate cortical bone. Furthermore, several sterilization methods have been investigated, including ethylene oxide, irradiation, hydrochloric acid decalcification, dimethyl sulfoxide, and freeze-drying for their ability to destroy the feline leukemia virus in the donor bone. All methods of sterilization failed to eliminate the virus. This is a significant finding because the feline leukemia virus is a retrovirus similar to human immunodeficiency virus.

Allograft is available in many preparations, however, the majority are composed primarily of cancellous or cortical bone. Cortical allografts provide immediate significant mechanical stability and structural support, while cancellous bone lends little mechanical stabilization on implantation but has a faster rate of incorporation. Cancellous allograft and particulate allograft preparations (cancellous or cortical) incorporate with new bone forming on the surface of trabeculae, with a large surface area available for new bone formation [5]. In contrast, cortical incorporation occurs slowly via a process of periosteal new bone formation around the allograft as an external callus derived from the host bone.

Particulate and structural grafts demonstrate significant differences in the histology of incorporation. Particulate grafts show more rapid and complete revascularization than structural grafts. Particulate bone remodels completely with time, while cortical bone remains a mixture of necrotic and viable bone. The process of creeping substitution also differs significantly between these forms of allograft with new bone formation occurring appositionally followed by resorption in cancellous bone. This process is reversed in cortical bone. These differences in biological capacity between graft types lead to significant differences in optimal clinical application.

Various clinical reports examining the performance of allograft for spinal fusion have been presented in the literature, but only a few have been prospectively designed and well conducted. The most favorable data are reported for one-level interbody fusion in the cervical spine with low rates of graft subsidence or resorption, but the union rate drastically drops in multilevel procedures. In the lumbar spine, cortical allograft is generally used for structural support (femoral rings) in combination with autogenous bone graft, showing only rare pseudarthrosis [2]. During recent years, the use of machine-threaded cortical allograft bone dowels or allograft interbody cages obtained from midshaft or diaphyseal bone have gained considerable popularity for anterior arthrodesis in the lumbar spine. Outcome data using these particular allografts are currently being collected. Several published reports have also addressed the use of allograft alone or mixed with autograft for posterior spinal fusion. When used in an instrumented thoracic spine the results are reported as favorable, in the lumbar spine it shows a lower fusion rate and higher resorption when compared with autograft.

2.3 CERAMICS AND BONE SUBSTITUTES

Because of the previously discussed problems associated with autograft and allograft material, there has recently been increased interest in biodegradable osteoconductive ceramic bone graft substitutes, which would be available in unlimited quantity, without donor-site complications or infectious risks.

For synthetic implants to be useful in vivo they must have certain properties:

- Compatibility with surrounding tissues.
- Chemical stability in body fluids.
- Compatibility of mechanical and physical properties.
- Ability to be produced in functional shapes.
- Ability to withstand the sterilization process.
- Reasonable cost of manufacturing.
- Reliable quality control [5].

The most common ceramic preparations that have been used in spinal reconstructive surgery include hydroxyapatite (HA) and tricalcium phosphate (TCP) [5]. They provide a biocompatible osteoconductive surface for bone regeneration and may contribute limited structural support. Other advantages of ceramic matrices include low immunogenicity and toxicity, stability at physiological pH levels, and the ability to withstand sterilization procedures without loosing structural integrity. Ceramics present with specific porosity, which may be artificially created and acts as a scaffold for further osteoblastic ingrowth. The unnatural pathway of a ceramic matrix with poorly interconnected porosity affects the ingrowing bone and retards the normal rate of bone healing and the remodeling process required to attain optimal mechanical strength.

The remodeling process depends on the biodegradability of the ceramic: nonresorbing materials may interfere with remodeling, be the locus of a mechanical stress riser, and impede the accretion of strength of the fusion mass. The various calcium phosphate ceramics differ with regard to their bioresorbability characteristics. Hydroxyapatite is relatively inert and biodegrades poorly, which may hinder remodeling, prolong the strength deficiency of new bone, and leave permanent stress risers in the fusion mass. Conversely, ceramic TCP undergoes biodegradation within the first 4–8 weeks of implantation, possibly too early for optimal fusion mass healing.

Natural ceramics derived from sea coral (Porites asteroides) are reported to have ideal pore size, interconnective porosity, and are structurally similar to cancellous bone. Composed of 97% calcium carbonate in the form of aragonite, coral undergoes a thermal reaction where calcium carbonates are transformed into HA. Coral is extremely biocompatible and has yielded promising results when it has been used to replace or augment autogenous bone graft [5], or as part of a composite with an osteoinductive bone protein. Despite these properties, the poor bioresorbability of the HA also applies to these natural ceramics resulting in poor bone remodeling.

While the organic phase of bone confers bone stiffness and compressive strength, ceramics are inherently brittle and susceptible to fracture with elasticity moduli significantly higher than those of cortical and cancellous bone and with low tensile strength. The overall crystalline structure and composition of bone apatite is similar, but not identical to that of HA, and this may explain differences observed in remodeling and resorption of ceramic preparations. Overall, more crystallization and higher mineral density yield greater

mechanical strength and lasting stability. In contrast, an amorphous simple preparation of calcium phosphate and calcium sulfate may also provide an osteoconductive matrix useful as a bone graft expender in spinal fusion, while retaining a rate of resorption that equals the rate of formation. Optimal remodeling of the spinal fusion mass is dependant upon biodegradability of the ceramic, which, depending on the crystalline structure and composition, may take from several months to several years. This problem seems to have been at least partially resolved by altering the processing of the coral with a partial thermoreaction where only 20% of the calcium carbonate is converted into HA [6].

Several preparations of ceramic matrices are currently available and they present different biological and mechanical characteristics [5]. Calcium sulfate and calcium phosphate are purely osteoconductive, with resorption properties closely matching the rate at which new bone is deposited. These materials are replaced by host bone through a process of creeping substitution. They exist in many different preparations including powders, pellets, putty, and injectable cement. Tricalcium phosphate has been used to fill bone defects. It has advantages similar to those of HA because it is biocompatible and bioabsorbable, but it is brittle and has very low impact resistance. Porous TCP has compressive and tensile strengths similar to, but lower than those of cancellous bone. The efficacy of these different ceramics in obtaining spinal arthrodesis has been studied in animal models and in selective clinical studies [7, 8]. Anterior interbody fusion in the thoracic spine of dogs has been analyzed using autologous tricortical iliac crest graft, HA ceramics, calcium carbonate, and a mixture of HA and TCP. Autogenous graft was shown to be the most effective material tested in these comparative studies [9]. Also, when combined with internal fixation, autogenous bone was significantly better than calcium carbonate ceramics.

Posterolateral intertransverse lumbar fusion was analyzed mainly in sheep and dogs [10–12]. Some authors demonstrated better results with autologous bone when compared with different ceramics, other authors showed similar results in terms of fusion rate when using coral porites (calcium carbonate) or a combination of HA and TCP.

Clinical data on the use of ceramics alone or combined with autogenous bone are limited. High fusion rates were reported in patients who had undergone cervical spinal fusion with interbody titanium cages filled with coral HA [13]. French authors have advocated the use of ceramics as graft extenders for autogenous bone in long instrumented fusions for deformities [14–16]. In a study of 12 adolescent patients, Passuti et al used a combination of HA and TCP with autogenous bone and found all patients to be clinically and radiologically fused [16]. Histology of specimens obtained from two of these patients indicated de novo bone ingrowth into the ceramic pores. However, these results must be interpreted with caution. In the cases of adolescent scoliosis treated with spinal fusion, the ceramic was used as a bone graft extender to supplement the local bone used as graft material. In addition, the patient population studied in this trial has a high propensity to healing, even without the addition of bone graft [16].

Although selective data from both animal and clinical studies seem favorable, the role of ceramic implants is still not well defined. Further discussion of their use as a complementary agent in composite form with osteoinductive growth factors are presently under investigation and are discussed in the next section.

2.4 DEMINERALIZED BONE MATRICES

The osteoinductive factors of bone are contained within the organic phase. While mineralized matrices have minimal osteoinductive activity, demineralized preparations have demonstrated a potent effect on the differentiation of osteoprogenitor cells into osteoblasts. A bone morphogenetic substance was first identified by Marshall Urist in his pioneering studies using soluble extracts from demineralized bone [17]. The demonstration of neoosteogenesis in response to ectopic submuscular implants of demineralized bone was a cornerstone in the further identification and cloning of bone morphogenetic proteins (BMPs).

The capacity of demineralized bone matrix (DBM) to induce new bone formation is now well established. The primary osteoinductive component of DBM consists of small amounts of glycoproteins in the organic phase of bone, the most important of which are the BMPs. The major pathway of neoosteogenesis induced by DBM is endochondral in subcutaneous and submuscular implants, and by direct induction of resident mesenchymal stem cells to osteoblasts and direct formation of bone without a cartilaginous intermediate in calvarial defects. This difference indicates the importance of the host environment in the process of osteogenesis induced by DBM.

Despite animal data suggesting a positive effect of DBM on spinal fusions, the clinical utilization of DBM in spinal arthrodesis has not demonstrated similar efficacy [18]. DBM preparations may be effective as graft extenders in the setting of limited autografts and as graft enhancers when comparing fusion quality to that of autograft alone, but results are equivocal. It is important to note that the osteogenic activity of a DBM preparation is highly dependent upon the type and specific preparation of bone used. The differing efficacy of DBM in spinal fusion demonstrated in the literature is likely to be a result of the different DBM preparations used [5].

In summary, despite good evidence for osteogenic activity in DBM, there is little evidence suggesting its effectiveness as a substitute for autogenous bone graft. There is an important variability in the osteoinductive capacity of different commercial demineralized bone graft preparations and this may contribute to variability in clinical experience. DBM offers no structural or mechanical stability independently of its carrier and does not appear to be a reliable substitute for autogenous bone graft. The material may have a role as a graft extender or as a supplement in hosts with compromised bone forming capacity.

2.5 OSTEOINDUCTIVE GROWTH FACTORS

Advances in cellular and molecular biology have led to the identification of specific cytokines that are active in mediating cellular activities including mitogenesis, anabolic activity, and cell differentiation. The ability to control cellular activity is a potentially powerful instrument in the management of orthopedic disorders and surgical reconstructions. Many growth factors and other cytokines have been shown to be osteoinductive in animal models. The growth factors that may enhance bone formation in vitro include insulin-like growth factor (IGF-1), acidic and basic fibroblast growth factors (aFGF, bFGF), platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β) [5, 19, 20].

Bone morphogenetic proteins are a subset of the TGF-β family and are the only cytokines that demonstrated a

capacity to induce new bone formation in vivo. Recent developments in recombinant techniques allow isolation of BMP in pharmacological quantities, in contrast to isolation through demineralization, in which less than 20 µg of osteoinductive material was extracted from 10 kg of bovine cortical bone. Clinical experience with BMP in spinal fusion studies suggested a valuable role as bone graft supplements or substitutes [21].

Composite grafts allow the combination of the osteoinductive and osteogenic capacities of growth factors or autogenous bone with the structural capacity of mineralized matrices. The ideal carrier for bone growth morphogenetic proteins has not been determined, but it would have reversible affinity to glycoprotein, structural characteristics, possibly including malleability or mechanical rigidity, limited immunogenicity and toxicity, and resorbability to permit complete replacement by bone. Inorganic carriers of BMP that have shown efficacy in promoting spinal arthrodesis include true bone ceramic (TBC), derived from sintered bovine bone, and hydroxyapatite TCP. Organic carriers include polylactic acid polymers (PLA), collagen and noncollagenous protein carriers, mineralized or demineralized bone matrix, and autograft. Their advantages include the capacity for chemical bonding to growth factors, and the provision of a biodegradable environment for new bone formation and graft incorporation. However, many organic carriers are weakly immunogenic and lack the osteoconductive function of inorganic bone cements. The structural capacity of inorganic cements is a further advantage of this carrier. Composite grafts offer potential for the design of bone graft substitutes that are specific for the structural and biological demands of the host, and it is likely that very different composites will be used for anterior interbody arthrodesis than for instrumented posterior fusion.

3 CONCLUSION

An understanding of the biological and structural characteristics of mineralized and demineralized bone matrix is necessary for their effective clinical application. The existing preparations have clear limitations in their clinical efficacy. Grafting materials and composites will continue to evolve for specific applications and their choice will be determined by the properties of the local host environment. The development of growth factors and other cytokines functioning as potent induction agents for neoosteogenesis offers tremendous potential for the design of composite materials providing osteoinduction, osteoconduction, and structural functions. Advances in tissue engineering and gene therapy will add a further osteogenic capacity to future bone graft substitutes.

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