ALTERATIONS OF THE SUBCHONDRAL BONE IN OSTEOCHONDRAL REPAIR – TRANSLATIONAL DATA AND CLINICAL EVIDENCE

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Abstract

Alterations of the subchondral bone are pathological features associated with spontaneous osteochondral repair and with articular cartilage repair procedures. The aim of this review is to discuss their incidence, extent and relevance, focusing on recent knowledge gained from both translational models and clinical studies of articular cartilage repair. Efforts to unravel the complexity of subchondral bone alterations have identified (1) the upward migration of the subchondral bone plate, (2) the formation of intralesional osteophytes, (3) the appearance of subchondral bone cysts, and (4) the impairment of the osseous microarchitecture as potential problems. Their incidence and extent varies among the different small and large animal models of cartilage repair, operative principles, and over time. When placed in the context of recent clinical investigations, these deteriorations of the subchondral bone likely are an additional, previously underestimated factor that influences the long-term outcome of cartilage repair strategies. Understanding the role of the subchondral bone in both experimental and clinical articular cartilage repair thus holds great promise of being translated into further improved cell- or biomaterial-based techniques to preserve and restore the entire osteochondral unit.

Keywords: Subchondral bone; microarchitecture; marrow stimulation; microfracture; subchondral drilling; abrasion arthroplasty.

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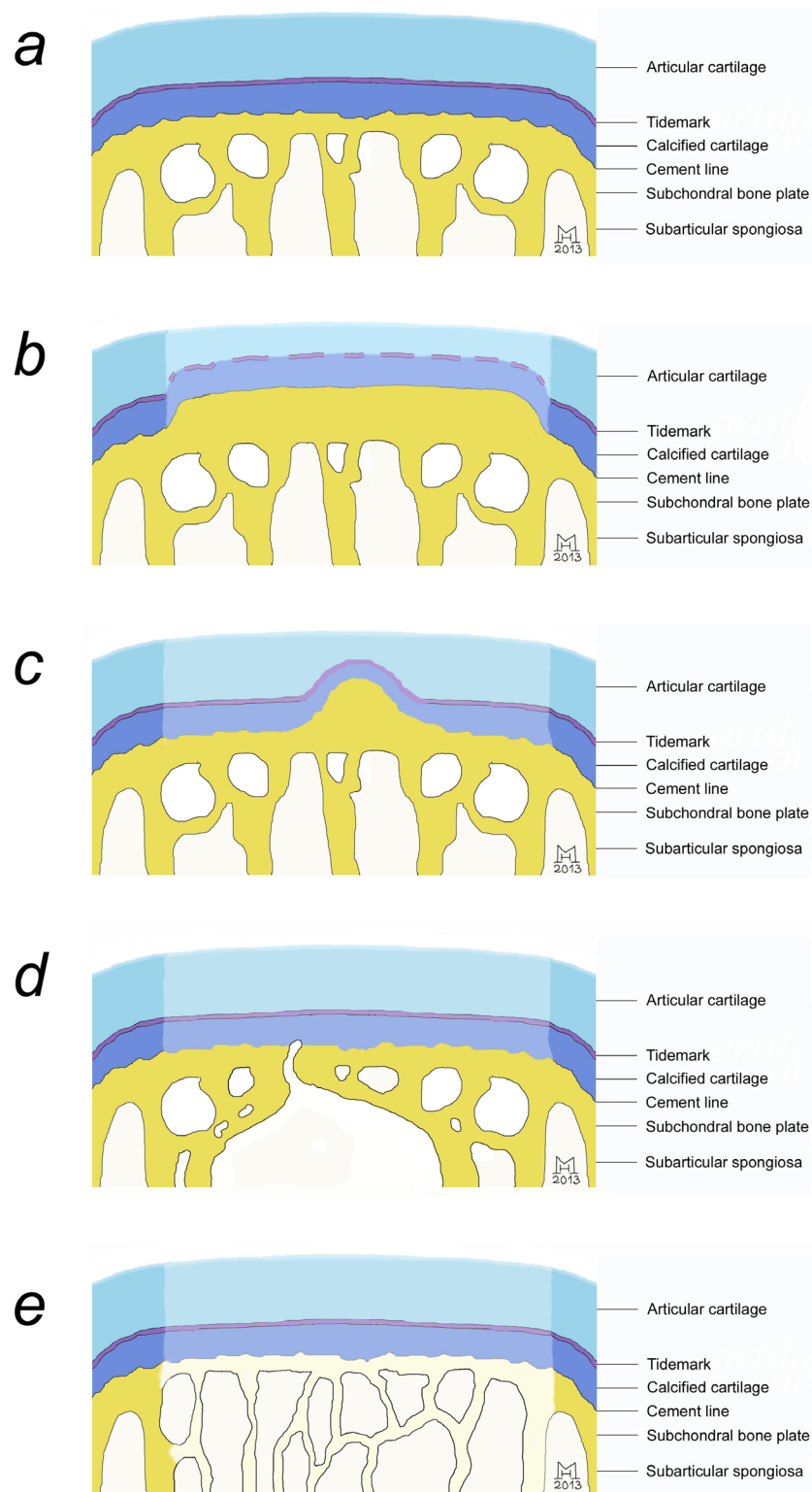   FAX Number: +49 6841 1624988
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Introduction

A complete restoration of the osteochondral unit is the goal of all articular repair techniques (Brittberg *et al.*, 1994; Johnson, 1986; Pridie, 1959; Steadman *et al.*, 2001). Traditionally, a focus was placed on the cartilaginous repair tissue, but it is now clear that complex structural changes of the subchondral bone are associated with spontaneous osteochondral repair and with the use of cartilage repair procedures (Madry *et al.*, 2010). They include (1) the upward migration of the subchondral bone plate, (2) the formation of intralesional osteophytes, (3) the appearance of subchondral bone cysts, and (4) the impairment of the osseous microarchitecture (Fig. 1). There is accumulating experimental evidence for these subchondral bone alterations in small (Aroen *et al.*, 2006; Chen *et al.*, 2009; Chen *et al.*, 2011a; Heir *et al.*, 2012; Marchand *et al.*, 2011; Marchand *et al.*, 2012; Nam *et al.*, 2004; Qiu *et al.*, 2003) and large preclinical animal models (Dorotka *et al.*, 2005; Frisbie *et al.*, 1999; Hanie *et al.*, 1992; Hoemann *et al.*, 2005; Howard *et al.*, 1994; Ishimaru *et al.*, 1992; Lane *et al.*, 2004; Orth *et al.*, 2012b; Vachon *et al.*, 1986) of cartilage defects. Supporting these findings, such pathological alterations were also reported in up to one third of patients treated with microfracture (Kreuz *et al.*, 2006; Mithoefer *et al.*, 2005; Saris *et al.*, 2009). Moreover, autologous chondrocyte implantation (ACI) for articular cartilage defects previously treated with marrow stimulation techniques has a three-fold higher failure rate than for untreated defects (Cole *et al.*, 2011; Minas *et al.*, 2009; Vasiliadis *et al.*, 2010). In addition, upward migration of the subchondral bone plate or the development of intralesional osteophytes might also occur spontaneously in large, full-thickness chondral lesions in patients (Henderson and La Valette, 2005), possibly playing a role in the degeneration of the cartilaginous repair tissue (Cole *et al.*, 2011; Minas *et al.*, 2009; Vasiliadis *et al.*, 2010). These studies point to the need to account for the complex role of subchondral bone alterations associated with osteochondral repair.

The aim of this review is to discuss the incidence, extent, and relevance of structural alterations of the subchondral bone, focusing on recent knowledge gained from both translational models and clinical studies of cell- and/or biomaterial-based procedures for cartilage repair.

Applied anatomy of the osteochondral unit

The subchondral bone is the layer of bone that lies immediately below the calcified zone of the articular cartilage (Fig. 1). Together with the cartilage, it forms the

Fig. 1. Synopsis of the structures constituting the normal osteochondral unit **(a)** and of the complex alterations of the subchondral bone during osteochondral repair (**b-e**; osteochondral repair tissue can be identified by its less intense colour). These alterations occur sporadically and are not ineluctable consequences of subchondral bone plate perforation. They may be categorised as **(b)** the generalised upward migration of the subchondral bone plate, **(c)** the formation of focal intralesional osteophytes, **(d)** the appearance of subchondral bone cysts, and **(e)** the impairment of the microarchitecture of the subchondral bone. Note the upward migration of the subchondral bone plate **(b)**, leading to a consecutive thinning of the articular cartilage layer and to an extension of the subchondral bone plate volume into the cartilaginous repair tissue. Intralesional osteophytes **(c)** are defined as focal newly formed bone located apical to the original cement line. An intralesional osteophyte can be either located in a central or peripheral location within the articular cartilage defect. The subchondral bone cyst **(d)** has its largest expansion within the subarticular spongiosa and is surrounded by a sclerotic rim. Note also the changes in the subchondral bone microarchitecture **(e)**, for example a generalised thinning of the subchondral bone plate, a reduced trabecular thickness, and an overall decreased subchondral bone volume.

osteochondral unit (Madry *et al.*, 2010). The basophilic line on histological sections that separates hyaline articular cartilage from the underlying calcified cartilage is called the tidemark (Broom and Poole, 1982), while the line that separates the calcified cartilage from the subchondral bone plate is called the cement line (Fig. 1) (Madry *et al.*, 2010). Two parts constitute the subchondral bone: The subchondral bone plate is composed of cancellous bone, consisting of bone plates which join together to enclose narrow intervening spaces. While denser than the subarticular spongiosa, the subchondral bone plate is relatively thin in normal human subchondral bone (Hunziker *et al.*, 2002). It is broader and more dense in osteoarthritic joints and in some animals (Frisbie *et al.*, 1999). The intervening spaces are gradually enlarged and become elongated in a direction parallel to the diaphysis in deeper regions of the subchondral bone, forming the subarticular spongiosa (Fig. 1) (Madry *et al.*, 2010). When the calcified cartilage layer is surgically removed (e.g. during the debridement of a cartilage defect prior to marrow stimulation), vascular canals of the subchondral region may be opened (Drobnic *et al.*, 2010).

Spontaneous and therapeutic osteochondral repair

Principles of spontaneous osteochondral repair
Osteochondral defects disrupt the structural integrity of the subchondral bone, but the natural history of the restoration of the subchondral bone plate in these defects is not well understood (Gomoll *et al.*, 2010b; Madry, 2010; Madry *et al.*, 2010; Orth *et al.*, 2013a; Pape *et al.*, 2010). Besides constituting the new cartilaginous repair tissue, mesenchymal cells in the deeper regions of the defect also differentiate into osteocytes (Jackson *et al.*, 2001; Shapiro *et al.*, 1993), resulting in the formation of immature bone that usually restores the original level of the subchondral bone in a distinct chronological order (Orth *et al.*, 2012a). Over time, however, this new subchondral bone may advance toward the joint space, and intralesional osteophytes might form (Orth *et al.*, 2012b).

Marrow stimulation techniques
Marrow stimulation procedures such as microfracture (Steadman *et al.*, 2001), subchondral drilling (Pridie, 1959) and abrasion arthroplasty (Johnson, 1986) are important and commonly applied first-line treatments for symptomatic small articular cartilage defects (Gomoll *et al.*, 2010a; Grana, 2000; Moran *et al.*, 2012; Safran and Seiber, 2010; Williams and Brophy, 2008). Altogether, these measures establish a communication of the cartilage defect with the bone marrow, either by focal perforation of the cement line with awls (microfracture) or drill bits (subchondral drilling) or by generalised abrasion to a maximal depth of 1-2 mm (Johnson, 1986; Johnson 2001) of the subchondral bone plate with round or cylindrical burrs (abrasion arthroplasty). These discrepancies in the surgical technique may yield differences in the subchondral bone response. Depending on the intra-articular location of the cartilage defect, one surgical treatment option might be superior to another with regard to technical feasibility. The common aim of these techniques is to allow mesenchymal cells from the underlying cavity to migrate into the defect (Gomoll *et al.*, 2010b; Tetteh *et al.*, 2012). Subsequently, the remodelling of the subchondral bone proceeds along with the induction of chondrogenesis and fibrocartilaginous repair (Shapiro *et al.*, 1993).

Autologous chondrocyte implantation
ACI is a two-stage surgical procedure, chiefly indicated for large focal cartilage defects (Brittberg *et al.*, 1994). After arthroscopic removal of a cartilage biopsy, the cultured chondrocytes are implanted during a second operation. While initially a periosteal flap was sewn over the defect to hold the cell suspension in place, chondrocytes are currently incorporated into biodegradable scaffolds (Batty *et al.*, 2011; Safran and Seiber, 2010). Similar to marrow stimulation procedures, the calcified cartilage layer at the bottom of the defect is completely removed prior to implantation. In contrast, however, care is taken not to induce bleeding from the subchondral bone. Interestingly, large animal models of cell-based cartilage repair show that besides the implanted chondrocytes, cells from the subchondral bone also participate in the formation of the repair tissue (Dell'Accio *et al.*, 2003; Jackson *et al.*, 2001).

Insights from translational models

Upward migration of the subchondral bone plate
Upward migration of the subchondral bone plate is defined as the expansion of the osteochondral junction above its original level with resulting elevation of the subchondral bone plate into the cartilaginous repair tissue (Orth *et al.*, 2012a) (Fig. 2).

In a rabbit model of spontaneous osteochondral repair, the subchondral bone reconstitution proceeded in a temporarily well-defined, distinct geometrical repair pattern (Orth *et al.*, 2012a). After six months, the subchondral bone is reconstituted to nearly normal levels. Then, the level of the new subchondral bone plate gradually advances above its native position and the cartilaginous repair tissue degrades (Orth *et al.*, 2012a; Qiu *et al.*, 2003) (Table 1) (Fig. 3). Of note, both processes advance at a different pace, as no statistical correlation was detected between articular cartilage repair and subchondral bone reconstitution (Orth *et al.*, 2012a; Orth *et al.*, 2013a).

A significant upward migration of the rabbit subchondral bone plate has also been observed 3 (Chen *et al.*, 2011a) or 9 months (Aroen *et al.*, 2006) after subchondral drilling and microfracture. In sheep, elevation of the subchondral bone plate beyond the former level of the tidemark was reported for chondral defects treated by microfracture after 1 year (Dorotka *et al.*, 2005). While this was not confirmed in another sheep model of drilled full-thickness cartilage defects after 6 months (Orth *et al.*, 2012b), in horses 50 % of drilled chondral defects exhibited elevation of the subchondral bone into the defect site (Shamis *et al.*, 1989) (Table 1).

In late stage osteoarthritis, the subchondral bone plate similarly advances towards the joint surface while the overlying cartilage layer narrows (Burr, 2004; Henrotin

et al., 2009; Lajeunesse *et al.*, 1999), possibly due to metaplasia of the deep layer of the articular cartilage (Shapiro *et al.*, 1993). Several investigations speculated that such an upward migration of the subchondral bone plate might be the primary cause for subsequent cartilage degeneration in osteoarthritis (Bullough and Jagannath, 1983; Green *et al.*, 1970; Jeffery, 1973; Radin *et al.*, 1991). Thickening of the subchondral bone plate increases its stiffness, and the thickness variation generated uneven pressure distribution as well as shear forces in the articular cartilage repair tissue that may initiate degeneration (Qiu *et al.*, 2003). In good agreement, Shahgaldi *et al.* (1991) emphasised that advancement of the subchondral hard tissue by only a few millimetres towards the articular surface is sufficient to cause injury to articular cartilage by loss of joint resilience at peak loading. Likewise, Qiu *et al.* (2003) demonstrated that in the presence of an advanced subchondral plate, repaired surface layers showed reduced Safranin O staining, increased separation splits at the boundary with neighbouring cartilage, softening, and eventual degradation.

Intralesional osteophytes

Intralesional osteophytes (Fig. 4) are defined as focal, newly-formed bone located apical to the original cement line and projected into the cartilaginous repair tissue layer (Cole *et al.*, 2011; Orth *et al.*, 2012b). Contrary to genuine chondro-osteophytes, which arise in the periosteum close to diarthrodial joints, intralesional osteophytes *per se* are not covered with a fibrocartilaginous cap (van der Kraan and van den Berg, 2007).

According to the observed articular cartilage degeneration overlying a generalised thickened subchondral
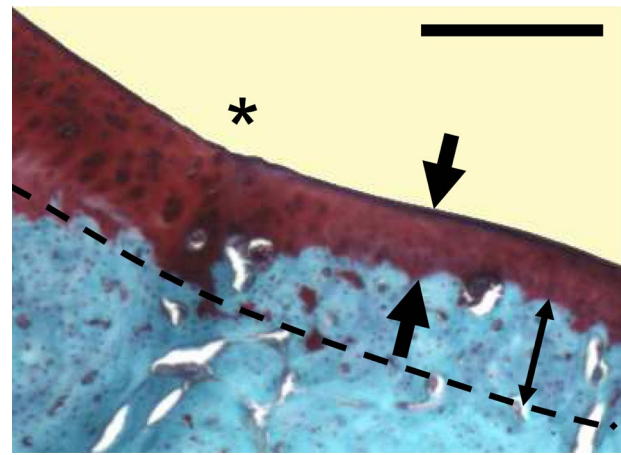

Fig. 2. Histological image of an untreated and spontaneously repaired osteochondral defect (diameter: 3.2 mm; depth: 5.0 mm) 1 year after its creation in the femoral trochlea of a 14-week old Chinchilla bastard rabbit stained with Safranin O/fast green. The integration site between repair tissue and native cartilage is still identifiable (star). The dotted line represents the normal position of the osteochondral junction. Thickening and upward migration of the subchondral bone plate (slim arrow) results in narrowing of the repair tissue layer covering the upwardly migrated subchondral bone (broad arrows). Scale bar: 1.0 mm.

bone plate, the development of focal intralesional osteophytes may also put the overlying cartilaginous repair tissue at further risk of degeneration (Fortier *et al.*, 2012). No data exist to date regarding the effect of focal intralesional osteophytes on cartilage degeneration.
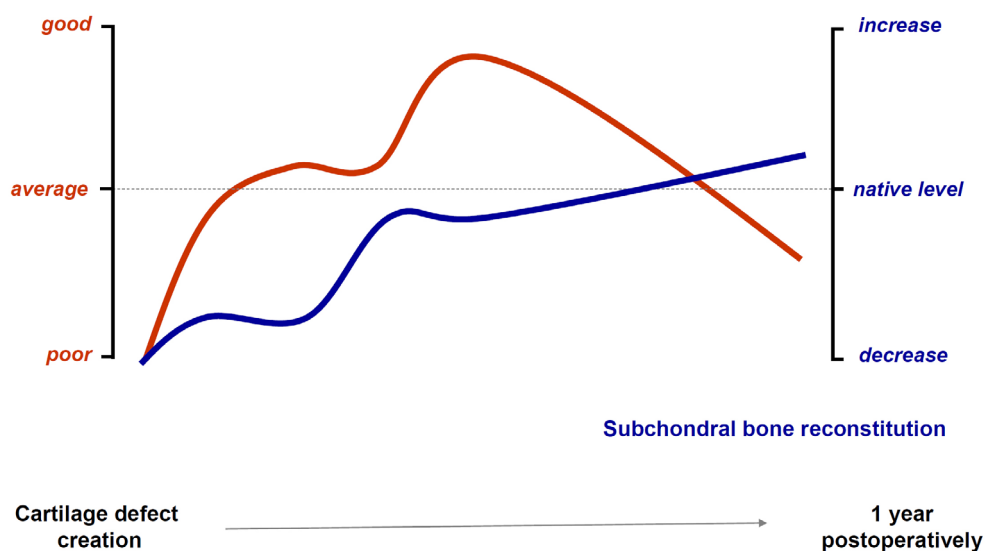

Fig. 3. Chronological sequence of the reconstitution of the subchondral bone and the quality of articular cartilage repair during the spontaneous repair of untreated osteochondral defects (diameter: 3.2 mm; depth: 5.0 mm) in the femoral trochlea of 14-week old Chinchilla bastard rabbits. Note that at early stages after osteochondral defect creation, both subchondral bone and articular cartilage repair are of inferior quality. Over a period of about six months, articular cartilage repair improves, and the subchondral bone is reconstituted to nearly normal levels. After one year, however, the subchondral bone advances above its native position and the repaired articular cartilage degrades in the lapine model. Potentially, inter-species differences in these chronological sequences may exist. Adapted from Orth *et al.* (2012a).

Table 1. Overview of reported subchondral bone alterations in experimental animal models of spontaneous osteochondral repair and following different articular cartilage repair procedures.

Pathology	Surgical procedure	Animal model	Detection method	Follow-up [months]	Number of animals	Incidence per defect	Distance	Reference
Upward migration	none (spontaneous osteochondral repair)	rabbit	histomorphometry	2	14	n.a.	− 0.79 mm	(Qiu *et al.*, 2003)
				8	7	n.a.	+ 0.13 mm	
	none (spontaneous osteochondral repair)	rabbit	histomorphometry	0.8	12	n.a.	− 0.57 mm	(Orth *et al.*, 2012a)
				12	10	n.a.	+ 0.19 mm	
	none (spontaneous chondral repair)	rabbit	histomorphometry	0.3	3	n.a.	− 0.09 mm	(Aroen *et al.*, 2006)
				0.5	6	n.a.	− 0.08 mm	
				9	8	n.a.	− 0.08 mm	
	none (spontaneous chondral repair)	sheep	histomorphometry	4	3	0%	n.a.	(Dorotka *et al.*, 2005)
				12	4	0%	n.a.	
	Drilling	rabbit	histomorphometry	0.3	3	n.a.	− 0.04 mm	(Aroen *et al.*, 2006)
				0.5	6	n.a.	+ 0.08 mm	
				9	8	n.a.	+ 0.07 mm	
	Drilling	rabbit	histomorphometry / μCT	3	8	38 - 50 %	n.a.	(Chen *et al.*, 2011)
	Drilling	sheep	μCT	6	19	0 %	n.a.	(Orth *et al.*, 2012b)
	Microfracture	rabbit	histomorphometry / μCT	3	8	50 %	n.a.	(Chen *et al.*, 2011)
	Microfracture	sheep	histomorphometry	4	3	0 %	n.a.	(Dorotka *et al.*, 2005)
				12	4	100 %	n.a.	
	Microfracture and ACI	sheep	histomorphometry	4	3	0 %	n.a.	(Dorotka *et al.*, 2005)
				12	4	100 %	n.a.	
Intralesional osteophyte	Drilling	sheep	histomorphometry	3	5	100 %	n.a.	(Ishimaru *et al.*, 1992)
	Drilling	sheep	μCT	6	19	26 %	n.a.	(Orth *et al.*, 2012b)
Cyst	none (spontaneous osteochondral repair)	horse	histomorphometry	12	10	n.a.	n.a.	(Howard *et al.*, 1994)
	none (spontaneous osteochondral repair)	horse	xeroradiography	4	3	n.a.	n.a.	(Hanie *et al.*, 1992)
				6	3	n.a.	n.a.	
	Drilling	rabbit	histomorphometry / μCT	3	8	41 %	n.a.	(Chen *et al.*, 2011)
	Drilling	sheep	histomorphometry	3	5	20 %	n.a.	(Ishimaru *et al.*, 1992)
	Drilling	sheep	μCT	6	19	63 %	n.a.	(Orth *et al.*, 2012b)
	Drilling	horse	histology	5	6	100 %	n.a.	(Vachon *et al.*, 1986)
	Microfracture	rabbit	histomorphometry / μCT	3	8	25 %	n.a.	(Chen *et al.*, 2011)
	Microfracture	sheep	histomorphometry	6	6	83 %	n.a.	(Hoemann *et al.*, 2005)
	Microfracture	horse	histomorphometry	4	5	0 %	n.a.	(Frisbie *et al.*, 1999)
				12	5	10 %	n.a.	
	Microfracture (chitosan-glycerol phosphate/blood)	sheep	histomorphometry	6	8	63 %	n.a.	(Hoemann *et al.*, 2005)

Positive values for the migration distance indicate an elevation of the subchondral bone plate above its native position (i.e. towards the joint line) while negative values indicate a position below its original location. n.a.: not available; μCT: micro computed tomography; ACI: autologous chondrocyte implantation.
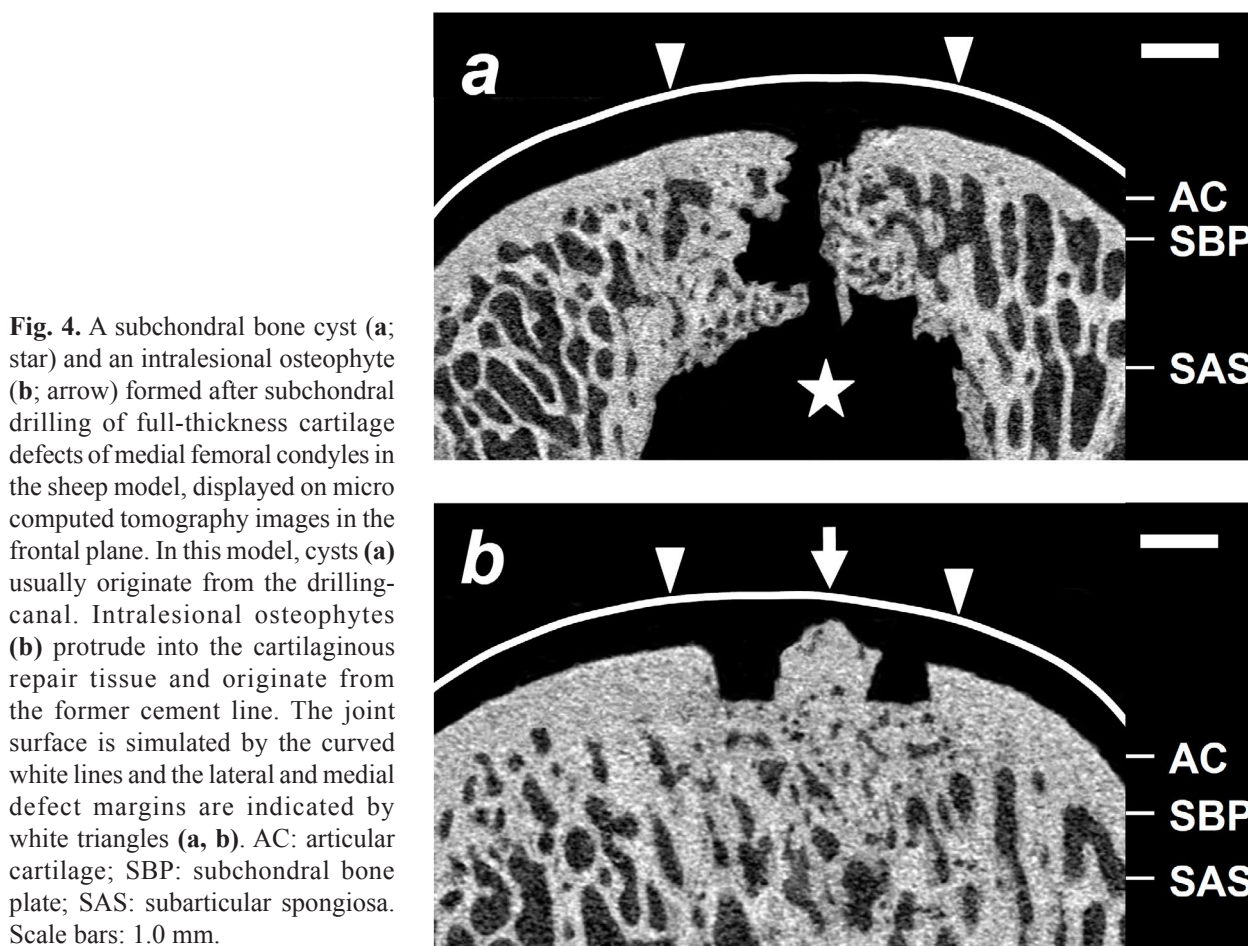
Fig. 4. A subchondral bone cyst (**a**; star) and an intralesional osteophyte (**b**; arrow) formed after subchondral drilling of full-thickness cartilage defects of medial femoral condyles in the sheep model, displayed on micro computed tomography images in the frontal plane. In this model, cysts **(a)** usually originate from the drilling-canal. Intralesional osteophytes **(b)** protrude into the cartilaginous repair tissue and originate from the former cement line. The joint surface is simulated by the curved white lines and the lateral and medial defect margins are indicated by white triangles **(a, b)**. AC: articular cartilage; SBP: subchondral bone plate; SAS: subarticular spongiosa. Scale bars: 1.0 mm.

However, it is possible, in theory, that a cartilaginous repair tissue connected to such bone overgrowth may be more stable. In the sheep model, intralesional osteophyte formation was observed after 3 months in all temporomandibular joints that had received perforation of the subchondral bone plate (Ishimaru *et al.*, 1992). Recent data revealed a 26 % incidence of intralesional osteophyte formation 6 months following drilling of full-thickness chondral defects of ovine medial femoral condyles with immediate and full weight-bearing postoperatively (Orth *et al.*, 2012b) (Table 1).

Subchondral bone cysts
Subchondral bone cysts (Fig. 4), an entity usually occurring in late-stage osteoarthritis (Pritzker *et al.*, 2006), are also reported in conjunction with marrow stimulation procedures in translational animal models.

Microfracture holes, drill holes, or the generalised thinning of the subchondral bone plate following abrasion arthroplasty may induce pathological bone resorption and subchondral cyst formation (Fig. 4) in horses (Frisbie *et al.*, 1999; Hanie *et al.*, 1992; Howard *et al.*, 1994; Vachon *et al.*, 1986) and rabbits (Chen *et al.*, 2011a) (Table 1). Ishimaru *et al.* (1992) observed subchondral cysts following perforation of the subchondral bone plate in the temporomandibular joint of sheep. By micro computed tomography (µCT) assessment of ovine cartilage defects treated by deep drilling, subchondral bone cysts were found in 63 % after 6 months (Orth *et al.*, 2012b). Hoemann

et al. (2005) reported on a decrease in subchondral cyst formation beneath microfracture-treated full-thickness chondral defects in sheep, by the additional transplantation of chitosan-glycerol phosphate-blood implants. Cysts have also been described in animal models following the implantation of proud (Pearce *et al.*, 2001) or unstable (Hurtig *et al.*, 2001; von Rechenberg *et al.*, 2003) osteochondral transplants (Heir *et al.*, 2012).

Changes in subchondral bone microarchitecture
The subchondral bone microarchitecture can be assessed by histomorphometric (Parfitt *et al.*, 1987) or three-dimensional radiological techniques (Feldkamp *et al.*, 1989; Marchand *et al.*, 2011; Orth *et al.*, 2012b).

The physiological reaction to marrow stimulation procedures is described as early bone resorption followed by a remodelling process resembling fracture healing (Heir *et al.*, 2012). The duration of this remodelling phase depends on the experimental model and ranges between 3 and 12 months (Dorotka *et al.*, 2005; Lane *et al.*, 2004; Nam *et al.*, 2004).

The work of Hoemann and Buschmann (Chen *et al.*, 2009; Chen *et al.*, 2011a; Chen *et al.*, 2011b; Marchand *et al.*, 2011; Marchand *et al.*, 2012) revealed that lapine subchondral bone surrounding drill holes remains morphologically intact at day 1, while empty osteocyte lacunae surround microfracture holes (Chen *et al.*, 2009). Regarding the drilling instrument itself, drills may be superior to Kirschner wires, which do not remove bone

debris – thus possibly impeding the migration of stem cells (Chen *et al.*, 2009). µCT showed that microfracture induces subchondral bone compaction with increased bone mineral density and trabecular thickness in rabbits, whereas drilling left a normal bone structure (Chen *et al.*, 2009). However, only 31 % of the original lapine subchondral bone plate remained 1 day after subchondral microdrilling (Marchand *et al.*, 2011). At 6 months, only partial restoration of the drilled subchondral bone plate was observed whereas the neighbouring subchondral plate had thickened, suggesting that drilling may also induce long-term changes in the adjacent rabbit subchondral bone (Marchand *et al.*, 2012). Interestingly, compared with shallow drilling (2 mm), deep drilling to 6 mm induced a larger volume of remodelling bone, which appeared beneficial for lapine cartilage repair after 3 months (Chen *et al.*, 2011a; Chen *et al.*, 2011b). Following an osteochondral injury, the microarchitecture of the subchondral bone can be improved by administration of bone-anabolic agents such as the 1-34 amino acid segment of the parathyroid hormone (Orth *et al.*, 2013a). Regarding the long-term effect of subchondral drilling on the microarchitecture of the subchondral bone in sheep, significantly reduced bone volume (BV/TV), trabecular thickness, and bone mineral density (BMD) were reported after 6 months (Orth *et al.*, 2012b). Interestingly, these changes were similar to patterns in osteoporosis and osteoarthritis: a decrease in BV/TV, BMD, and trabecular thickness is a typical finding in sheep (Mastbergen *et al.*, 2008; Wu *et al.*, 2008), dogs (Sniekers *et al.*, 2008), or goats (Siu *et al.*, 2004) suffering from osteoporosis or osteoarthritis. Of note, the relationship between repair bone architecture and the quality of the cartilage repair tissue is still the object of investigation by many different research groups using distinct approaches.

Relevance of subchondral bone alterations for experimental cartilage repair

The histological aspect of the fibrocartilaginous repair tissue in marrow-stimulated cartilage defects is well described (Fortier *et al.*, 2012; Frisbie *et al.*, 1999; Furukawa *et al.*, 1980; Goebel *et al.*, 2012; Jackson *et al.*, 2001; Shapiro *et al.*, 1993). In rabbits, the access of bone marrow elements to the lesions by microfracture (Heir *et al.*, 2012) or drill holes (Aroen *et al.*, 2006) caused an increased filling after 9 months, when compared to defects lacking such access (Shamis *et al.*, 1989). These data were confirmed in the sheep model at 1 year (Dorotka *et al.*, 2005). Besides quantity, the quality of the repair tissue may further be enhanced by implantation of chitosan-glycerol phosphate (Chevrier *et al.*, 2007; Hoemann *et al.*, 2005), thrombin-solidified chitosan (Marchand *et al.*, 2012), or cell-seeded collagen matrices (Dorotka *et al.*, 2005).

Comparing subchondral drilling with the microfracture technique, Chen *et al.* (2009) found a similar repair of full-thickness chondral defects in the lapine trochlear groove of rabbits. Interestingly, deep drilling (6 mm) elicited a greater defect fill, increased glycosaminoglycan and type II collagen content, and reduced type I collagen content of the repair tissue than shallow drilling (2 mm) (Chen *et al.*, 2009).

The success of marrow stimulation for chondral lesions depends on the meticulous removal of the calcified cartilage layer (which is best achieved by open debridement with a curette) (Drobnic *et al.*, 2010), improving the arthroscopic (4 months) and macroscopic (1 year) aspect of equine repair tissues, although not affecting histological, biochemical or imaging analyses (Frisbie *et al.*, 2006). Interestingly, no effect of drill hole diameter (0.5 *versus* 0.9 mm) on the quality of cartilage repair was seen 6 months after subchondral drilling in rabbits (Marchand *et al.*, 2012).

Clinical evidence of subchondral bone alterations

Upward migration of the subchondral bone plate
Particularly in clinical investigations, with lower image resolution techniques compared to experimental studies (Menetrey *et al.*, 2010), differentiation between laminar upward migration of the entire subchondral bone plate (Fig. 5) and selective, localised formation of intralesional osteophytes (Fig. 6) is difficult to document. Several authors refer to both phenomena by the term "osseous overgrowth" (Kreuz *et al.*, 2006; Minas *et al.*, 2009; Mithoefer *et al.*, 2005). Mithoefer *et al.* (2005) observed, by magnetic resonance imaging (MRI), thickening and
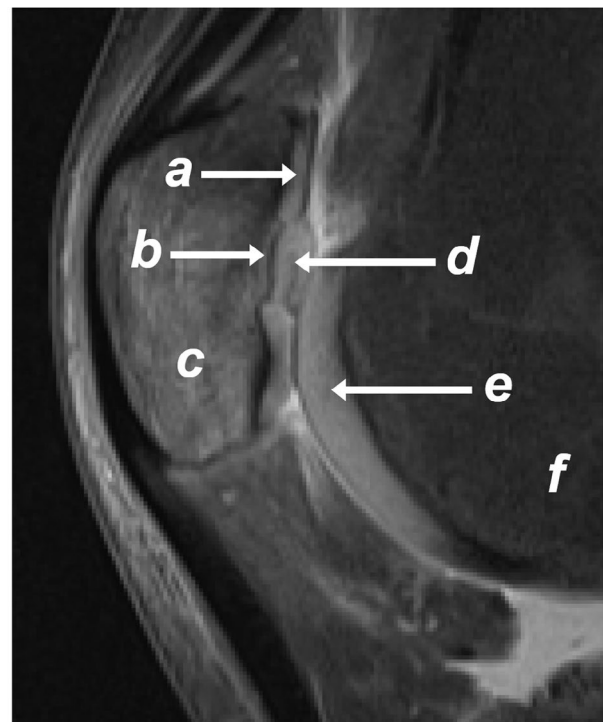

Fig. 5. Magnetic resonance image of the right knee joint of a 21-year-old man 6 months following arthroscopic abrasion arthroplasty of a chondral defect at the patella. Normal articular cartilage neighbouring the defect site can be identified proximal (a) and distal to the defect on the dorsal surface of the patella (c) as well as on the condylar circumference of the distal femur (f). Note the significant upward migration of the subchondral bone plate within the former defect area (b), which is covered by an irregular cartilaginous repair tissue (d) protruding into the patellofemoral joint.

Table 2. Overview of reported subchondral bone alterations following the clinical use of articular cartilage repair procedures.

Pathology	Surgical index procedure	Detection method	Follow-up [months]	Number of patients	Incidence per defect	Reference
Upward migration	Microfracture	MRI	12	24	25.0 %	(Mithoefer *et al.*, 2005)
	Microfracture	MRI	36	44	52.0 %	(Saris *et al.*, 2009)
	ACI	MRI	36	41	25.0 %	(Saris *et al.*, 2009)
	ACI	MRI	36	179	33.5 %	(Henderson and La Valette, 2005)
Intralesional osteophyte	Drilling (type I/III collagen, PRP gel)	MRI	24	5	60.0 %	(Dhollander *et al.*, 2011)
	Microfracture	MRI	36	70	27.1 %	(Kreuz *et al.*, 2006)
	Microfracture	MRI	15	80	48.8 %	(Brown *et al.*, 2004)
	Microfracture	MRI	0.8	13	0.0 %	(Cole *et al.*, 2011)
			6	13	53.8 %	
			12	13	69.2 %	
			24	8	75.0 %	
	ACI	MRI	13	34	23.3 %	(Brown *et al.*, 2004)
	ACI	MRI	155	31	63.9 %	(Vasiliadis *et al.*, 2010)
Cyst	Microfracture	MRI	0.8	13	0.0 %	(Cole *et al.*, 2011)
			6	13	15.4 %	
			12	13	38.5 %	
			24	8	37.5 %	
	ACI	MRI	155	31	38.8 %	(Vasiliadis *et al.*, 2010)

MRI: magnetic resonance imaging; ACI: autologous chondrocyte implantation; PRP: platelet-rich plasma.
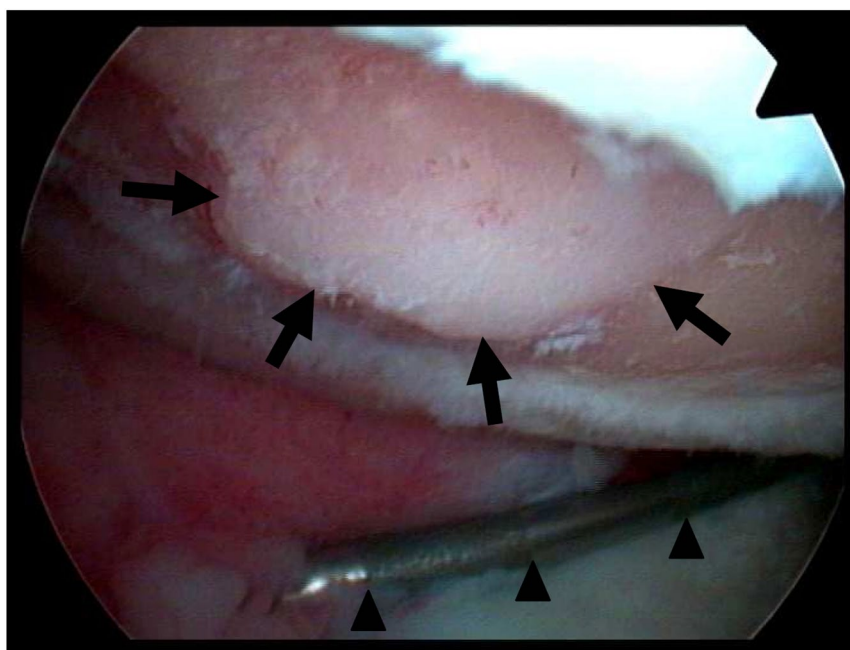
Fig. 6. Arthroscopic view of a large intralesional osteophyte (pointed at by arrowheads) which developed spontaneously without prior surgical treatment within a full-thickness chondral defect in the weightbearing area of the medial femoral condyle in a 43-year-old man. A marking on the calibrated arthroscopic probe (indicated by black triangles) measures 5.0 mm.

upward migration of the apical border of the subchondral bone plate in 25 % of patients treated with the microfracture technique at a mean follow-up of 41 months (Table 2). Saris *et al.* (2009) assessed structural MRI outcome parameters 3 years after microfracture using the two-dimensional MOCART score (Marlovits *et al.*, 2004). Defects treated by microfracture developed a progressive elevation of the subchondral bone plate in 52 % of patients.

Generalised elevation of the subchondral bone plate may eventually predispose for the development of osteoarthritis (Potter and Chong, 2009). The cartilaginous repair tissue forms on a comparably thickened, prominent and stiff subchondral bone plate (Simon, 1970), which alters the biomechanical properties of the repair cartilage, such as load distribution throughout the joint (Guettler *et al.*, 2004; Simon, 1970). This may possibly result in an extension of the defect and ultimately osteoarthritic joint degeneration (Minas *et al.*, 2009).

Following ACI, Minas *et al.* (2009) reported on an upward migration of the subchondral bone plate in 25 % of defects at 3 years postoperatively. In agreement, Henderson and La Valette (2005) described an upward migration of the subchondral bone plate in 34 % of chondral defects as detected by MRI 3 years after ACI (Table 2). Such upward migration was detected in 45 % of defects located at the lateral femoral condyle, in 34 % of defects at the medial femoral condyle, in 29 % of defects located at the trochlea and in 14 % of retropatellar defects. Interestingly, there was a significant association between upward migration of the subchondral bone plate and a larger defect size (mean 3.4 cm^2) as well as with location at the lateral femoral condyle (Henderson and La Valette, 2005).

Intralesional osteophytes
Often difficult to distinguish from generalised thickening of the subchondral bone plate, intralesional osteophytes are only seldom described in clinical investigations (Brown *et al.*, 2004; Cole *et al.*, 2009; Dhollander *et al.*, 2011; Kreuz *et al.*, 2006). Cole *et al.* (2011) did not observe intralesional osteophytes at 3 weeks by MRI assessment, but in 54 % of defects at 6 months, in 69 % at 1 year, and in 75 % at 2 years after microfracture (Table 2). Kreuz *et al.* (2006) reported on the formation of intralesional osteophytes in 27 % of patients 3 years after microfracture, as detected by MRI evaluation. A propensity for bony overgrowth, resulting in intralesional osteophytes in 49 % of patients – with concomitant loss of adjacent cartilage, was also observed by Brown *et al.* (2004) at a mean follow-up period of 15 months after microfracture treatment. Dhollander *et al.* (2011) treated chondral defects of the patella by subchondral drilling in combination with the transplantation of type I/III collagen membranes and a platelet-rich plasma gel. MRI assessment at 2 years postoperatively revealed intralesional osteophytes in 60 % of patients (Dhollander *et al.*, 2011).

Subsequent to ACI, intralesional osteophytes were also detected by MRI in 23 % of patients 13 months postoperatively (Brown *et al.*, 2004). Remarkably, almost one third (29 %) of these ACI-treated patients presenting with intralesional osteophytes had undergone a previous marrow stimulation procedure (Brown *et al.*, 2004). At a longer follow-up after ACI (mean 12.9 years), Vasiliadis *et al.* (2010) found intralesional osteophytes in 64 % of defects. Here again, previous affection of the subchondral bone (prior marrow stimulation procedures; history of osteochondrosis dissecans) was evident for 70 % of intralesional osteophytes.

Subchondral bone cysts
Following microfracture treatment, Cole *et al.* (2011) detected subchondral cysts beneath the articular cartilage repair tissue by MRI assessment in 15 % of treated defects at 6 months, in 39 % at 1 year and in 38 % at 2 years (Table 2). Interestingly, no cysts were found at 3 weeks

postoperatively. These findings suggest that subchondral bone cysts do not occur in the first weeks after articular cartilage resurfacing procedures, but are detectable as early as 6 months postoperatively.

At a long-term follow-up between 9 and 18 years after ACI, subchondral cysts were reported in 39 % of defects (Vasiliadis *et al.*, 2010).

Changes in subchondral bone microarchitecture
In patients with a history of previous marrow stimulation, several studies have addressed the microscopic structure of the articular cartilage repair tissue by evaluating biopsies taken during second-look arthroscopies (Bae *et al.*, 2006; Gobbi *et al.*, 2005; Gudas *et al.*, 2005; Knutsen *et al.*, 2007; Knutsen *et al.*, 2004; Mainil-Varlet *et al.*, 2010; Saris *et al.*, 2009). However, no investigation has histologically examined the microarchitecture of the subchondral bone so far. An entire osteochondral biopsy would be necessary for this purpose, but for ethical considerations can only infrequently be obtained from repaired defect sites in the clinical setting. In theory, such an osteochondral biopsy may be subjected to non-invasive µCT for subchondral bone analysis prior to histological processing. As the resolution power of up-to-date µCT scanners is below 1.0 µm (Orth *et al.*, 2012b), while core biopsies are usually obtained using 8-13 G Jamshidi needles (diameter 1.8-3.3 mm) (Wei *et al.*, 2003), the technical prerequisites that would allow for the assessment of subchondral bone microarchitecture in such biopsies are already in place. In contrast, for both ethical and technical reasons, the use of high resolution µCT or MRI scanners for *in situ* analysis of the subchondral bone is still not realisable in patients.

Relevance of subchondral bone alterations for cartilage repair and joint function
Regarding the clinical long-term results of marrow stimulation techniques, good to excellent results are reported in 60-80 % of patients (Basad *et al.*, 2010; Knutsen *et al.*, 2004; Knutsen *et al.*, 2007; Kon *et al.*, 2009; Mithoefer *et al.*, 2009; Mithoefer *et al.*, 2005; Steadman *et al.*, 2003; Visna *et al.*, 2004). Clinical improvement is achieved as early as 6 months, with the largest improvement occurring during the first 18-24 months (Kreuz *et al.*, 2006; Mithoefer *et al.*, 2009). Studies investigating the repair tissue by MRI showed a high variability (Hayter and Potter, 2011): The grade of defect filling ranged between 18 and 95 % after microfracture treatment of chondral defects (Bachmann *et al.*, 2004; Brown *et al.*, 2004; Gudas *et al.*, 2005; Mithoefer *et al.*, 2005; Ramappa *et al.*, 2007), despite the good correlation of this parameter with the clinical outcome (Kreuz *et al.*, 2006; Mithoefer *et al.*, 2005). Excellent or good MRI results with regard to the presence of subchondral cysts, thickness of the repair tissue, reconstitution of joint congruency (Gudas *et al.*, 2005) or when applying the MOCART system for evaluation purposes (Von Keudell *et al.*, 2011) were found in 8-57 % of treated defects. Mithoefer *et al.* (2005) reported on pathological MRI signals within the repair tissue in 96 %, subchondral oedema in 71 %, and insufficient integration of the repair tissue in 92 % of defects two years after microfracture.

In second-look arthroscopies, following marrow stimulation procedures (Bae *et al.*, 2006; Gill, 2000; Gobbi *et al.*, 2005; Gudas *et al.*, 2005; Knutsen *et al.*, 2004; Knutsen *et al.*, 2007; Mainil-Varlet *et al.*, 2010; Nehrer *et al.*, 1999; Ramappa *et al.*, 2007; Saris *et al.*, 2009; Saw *et al.* 2011), defects are usually well covered with fibrocartilaginous repair tissue at different follow-up periods (Bae *et al.*, 2006; Gobbi *et al.*, 2005; Ramappa *et al.*, 2007; Saw *et al.*, 2011). However, repaired defects only reach average macroscopic grading scores (Bae *et al.*, 2006; Gudas *et al.*, 2005; Knutsen *et al.*, 2004; Riyami and Rolf, 2009). Describing the quality of the cartilage repair tissue more precisely, Gill (2000) reported on fibrillated surfaces in 16 % and fragmented filling in 18 % of defects treated by microfracture 12 months postoperatively. Compared with ACI, microfracture resulted in significantly lower concentrations of type II collagen and proteoglycan in the repair cartilage (Saris *et al.*, 2009). In 1999, Nehrer, Spector and Minas analysed the composition of the reparative tissue from full thickness chondral defects retrieved during revision surgery in 12 patients with failed abrasion arthroplasty. Here, the histological appearance was that of fibrous, spongiform tissue comprising type I collagen in 22 % of the histological cross sectional area, degenerating hyaline tissue in 30 % and fibrocartilage with positive type II collagen staining in 28 % (Nehrer *et al.*, 1999). In good agreement, the repair tissue of patients with failed microfracture or subchondral drilling was fibrocartilaginous and hypercellular 4-19 months after marrow stimulation (Kaul *et al.*, 2012). Interestingly, the subchondral bone beneath this repair tissue was incompletely restored and a new tidemark was absent (Kaul *et al.*, 2012), indicating the lack of a complete regeneration of the osteochondral unit. However, these structural changes still need to be correlated to the clinical outcome of patients in further investigations.

Compared with marrow stimulation procedures, changes to the subchondral bone are less frequently reported following ACI in clinical investigations (Brown *et al.*, 2004; Henderson and La Valette, 2005; Saris *et al.*, 2009; Vasiliadis *et al.*, 2010). Although Saris *et al.* (2009) and Henderson and La Valette (2005) reported on a lower incidence of subchondral bone plate upward migration following ACI (25 and 34 %, respectively; Table 2) compared with microfracture (52 %) at 3 years postoperatively, Vasialiadis *et al.* (2010) found similar high incidences of intralesional osteophytes and subchondral bone cysts following ACI up to 18 years postoperatively. This suggests that the reported changes in the subchondral bone are comparable between these two different therapeutic strategies in patients.

Possible aetiopathologies of subchondral bone alterations

No hypothesis has yet been provided to explain the pathological changes in the subchondral bone. Possible aetiopathologies, alone or in synergism, include, but may not be limited to, impaired bone and articular cartilage regeneration processes, pathological structural

consequences of altered biomechanical loading, disturbed mechanisms of articular cartilage-subchondral bone crosstalk, and pathological vascularisation or angiogenesis.

For example, exposure or perforation of the subchondral bone plate may start the process of bone regeneration, which may extend into the region where the articular cartilage is supposed to be. Similar to the physiological process of endochondral bone formation, bone marrow-derived mesenchymal stem cells (MSCs) and/or chondrocytes may undergo hypertrophy in the deeper regions of the repair tissue, and are then replaced by bone, leading to the pathological formation of osseous tissue within the cartilaginous compartment (Studer *et al.*, 2012). This theory is supported by the presence of type I and type X collagen in the cartilaginous repair tissue (Kaul *et al.*, 2012). Many factors, among which parathyroid hormone (PTH [1-34]) (Orth *et al.*, 2013a), Sox9 (Cucchiarini *et al.*, 2013), enamel matrix derivative (Kiss *et al.*, 2012), bone morphogenetic proteins and their inhibitors (Rosen, 2006; Ruschke *et al.*, 2012), IGF-I (Kim *et al.*, 2013; Madry *et al.*, 2005), or TGF-β (Li *et al.*, 2013; Re'em *et al.*, 2012; Serra *et al.*, 1997), have either been shown or suggested to affect structural patterns of subchondral bone repair in animal models of osteochondral defects *in vivo*. An imbalance between them may impair the regulation of osteochondral repair. Such deterioration in the crosstalk between cartilage and bone during osteochondral repair (Funck-Brentano and Cohen-Solal, 2011) may additionally be ascribed to hypoxia (Studer *et al.*, 2012) or altered signalling pathways, potentially involving for example the hedgehog family (Rockel and Alman, 2011), the PTH-receptor (Orth *et al.* 2013a), Wingless/Int (Wnt) (Blom *et al.*, 2010), interleukins (Greenfield *et al.*, 1996), the receptor activator of NF-kappa B ligand (RANKL) (Kwan Tat *et al.*, 2008), or osteoprotegerin (Palmqvist *et al.*, 2002). In addition, vascularisation and angiogenesis may play a role in this process (Gerber and Ferrara, 2000), with different angiogenic (and anti-angiogenic) factors such as VEGF (Gerber *et al.*, 1999; Joyce *et al.*, 1991) being involved. Interestingly, work of Ernst Hunziker showed that obstruction of blood vessel upgrowth from the subchondral bone into the articular cartilage repair tissue significantly reduced subchondral bone plate advancement (Hunziker and Driesang, 2003; Hunziker *et al.*, 2001).

Furthermore, since the quality of the cartilaginous repair tissue is persistently inferior to normal and therefore cannot redistribute load as effectively as normal articular cartilage, biomechanical stresses imposed on the subchondral bone are consecutively increased (Chen *et al.*, 2011a; Qiu *et al.*, 2003). As bone remodels in response to the increased load (Yokota *et al.*, 2011), this response may be inappropriate, inducing either subchondral bone plate migration and intralesional osteophytes, e.g. by metaplasia of the deep layer of the articular cartilage (Shapiro *et al.*, 1993), or impairment of the subchondral bone microarchitecture and the appearance of subchondral bone cysts (Gomoll *et al.*, 2010b; Vasara *et al.*, 2004).

Interestingly, recent work has failed so far to reveal correlations between articular cartilage and subchondral bone repair (Orth *et al.*, 2012a; Orth *et al.*, 2013a), suggesting that both tissues may repair independently.

Conclusion

Structural alterations of the subchondral bone are associated with spontaneous osteochondral repair and articular cartilage repair procedures in both translational animal models and patients. Experimental data confirm a relevant upward migration of the perforated subchondral bone plate over time. In these models, articular cartilage repair and subchondral bone reconstitution proceed at a different pace, as indicated by the lack of a significant correlation among them (Orth *et al.*, 2012a; Orth *et al.*, 2013a). The subchondral bone plate surrounding treated defects thickened following subchondral drilling (Marchand *et al.*, 2012), suggesting that marrow stimulation may also induce long-term changes in the adjacent subchondral bone.

Clinical investigations show that marrow stimulation techniques exhibit promising clinical results, especially within the first 2 years. According to the experimental findings, marrow stimulation has been associated with the upward migration of the subchondral bone plate, intralesional osteophytes, or bone cysts in a large number of patients. It may be speculated whether these changes play a role in the degeneration and failure of the repair tissue. In good agreement, alterations of the subchondral bone have also been described following ACI, albeit less frequently reported. Here, their incidence is comparable to marrow stimulation techniques, suggesting that none of these different therapeutic approaches is superior with regard to subchondral bone pathologies. Strategies to diminish these subchondral bone alterations are needed, e.g. by optimising different perforation or debridement techniques or postoperative rehabilitation regimes (Anderson and Smith, 2009).

The frequently observed subchondral bone cysts following marrow stimulation are often unrelated to symptoms in patients. Yet, their clinical relevance still needs to be evaluated in more detail (Cox *et al.*, 2011). Regarding the radiographic analysis of the repair process, μCT revealed that marrow stimulation procedures deteriorate the microarchitecture of the entire osteochondral unit for an extended postoperative period. It remains to be elucidated whether pharmacological therapies aimed at counterbalancing these induced pathological changes of the subchondral bone may improve the clinical outcome of marrow stimulation techniques.

Altogether, these emerging experimental and clinical data suggest that a deterioration of the underlying subchondral bone plate and subarticular spongiosa might be an additional, previously underestimated factor that influences the long-term outcome of cartilage repair strategies. These findings are highly relevant for translational cartilage repair models employing cell- or biomaterial-based approaches, since osteochondral repair not only affects the articular cartilage, but also the subchondral bone. A deeper comprehension of the complex role of the subchondral bone in both experimental and clinical articular cartilage repair holds great promise of being translated into further improved cell- or biomaterial-based techniques to preserve and restore the entire osteochondral unit.

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Discussion with Reviewers

Reviewer I: In translational studies, what set of outcome parameters would you recommend to be implemented for assessing the pathological changes in the subchondral bone?

Authors: In translational animal studies, the upward migration of the subchondral bone plate can be assessed by histomorphometrical measurements. Standardised methods to determine the migration of the subchondral bone plate on histological sections have been described (Orth *et al.*, 2012a; Qiu *et al.*, 2003). For the formation of subchondral bone cysts, the group of Hoemann *et al.* (2005) has established a scoring system to evaluate their formation and severity. With special regard to the formation of subchondral bone cysts following marrow stimulation procedures and their differentiation to lacunae of the subarticular spongiosa, cysts are defined as having a minimum diameter of at least triple the diameter of the perforation instrument (microfracture awl, subchondral bone drill) (Orth *et al.*, 2012b). The incidence of subchondral bone cysts and intralesional osteophytes can be assessed and quantified by both micro-CT and histomorphometry. We have previously categorised intralesional osteophytes as being either central or peripheral, depending whether they are located between subchondral bone perforations (microfracture holes or drill holes) or between perforations and the defect border (Orth *et al.*, 2012b). To assess the impairment of the osseous microarchitecture, micro-CT as well as histomorphometrical evaluation are suitable. In our opinion, the most crucial parameters for both evaluation techniques are bone mineral density (BMD), bone volume fraction (BV/TV), bone surface/volume ratio (BS/BV), bone surface density (BS/TV), cortical thickness (Ct.Th), trabecular thickness (Tb.Th), and trabecular separation (Tb. Sp) (Feldkamp *et al.*, 1989; Orth *et al.*, 2013a; Orth *et al.*, 2012b). However, these measures are more difficult to be applied in clinical studies.

Reviewer II: What mechanisms do the authors believe are responsible for the 'upward migrating' of the subchondral bone plate?

Authors: We believe that endochondral ossification is a major mechanism that may be responsible for the upward migration of the subchondral bone plate as well as for the formation of intralesional osteophytes. Similar to

the physiological process of endochondral ossification of the growth plate (Gerber and Ferrara, 2000; Studer *et al.*, 2012), chondrocytes may undergo hypertrophy in the deeper regions of the repair tissue, and are then replaced by bone, leading to the pathological formation of osseous tissue within the cartilaginous compartment. This theory is supported by the presence of type I and type X collagen in the cartilaginous repair tissue (Kaul *et al.*, 2012). Vascularisation and angiogenesis play a key role in this process (Gerber and Ferrara, 2000). Furthermore, biomechanical stresses imposed on the subchondral bone within the defect are consecutively increased (Chen *et al.*, 2011; Qiu *et al.*, 2003). As bone remodels in response to the increased load (Yokota *et al.*, 2011), this response may be inappropriate, inducing subchondral bone plate migration.

Reviewer II: What cells are likely to be involved in subchondral bone changes and what are the driving forces?
Authors: Cells involved in the formation of intralesional osteophytes or in the upward migration of the subchondral bone plate are most likely osteoblasts from the subchondral bone plate as well as bone-marrow derived stems cells which gain access to the defect (Jackson *et al.*, 2001; Shapiro *et al.*, 1993). Furthermore, cells from the synovial lining may also contribute to the repopulation of the cartilage defects (Hunziker and Rosenberg, 1996). All of these cell populations may differentially express various factors, among which parathyroid hormone (PTH [1-34]) (Orth *et al.*, 2013a), Sox9 (Cucchiarini *et al.*, 2013), enamel matrix derivative (Kiss *et al.*, 2012), bone morphogenetic proteins and their inhibitors (Rosen, 2006; Ruschke *et al.*, 2012), IGF-I (Kim *et al.*, 2013; Madry *et al.*, 2005), or TGF-β (Li *et al.*, 2013; Re'em *et al.*, 2012; Serra *et al.*, 1997) have either been shown or suggested to affect structural patterns of subchondral bone repair in animal models of osteochondral defects *in vivo*. An imbalance between them may impair the regulation of osteochondral repair. Such deterioration in the crosstalk between cartilage and bone during osteochondral repair (Funck-Brentano and Cohen-Solal, 2012) may additionally be ascribed to hypoxia (Studer *et al.*, 2012) or altered signalling pathways, potentially involving for example the hedgehog family (Rockel and Alman, 2011), the PTH-receptor (Orth *et al.* 2013a), Wingless/Int (Wnt) (Blom *et al.*, 2010), interleukins (Greenfield *et al.*, 1996), the receptor activator of NF-kappa B ligand (RANKL) (Kwan Tat *et al.*, 2008), or osteoprotegerin (Palmqvist *et al.*, 2002). In addition, vascularisation and angiogenesis may play a role in this process (Gerber and Ferrara, 2000), with different angiogenic (and anti-angiogenic) factors such as VEGF (Gerber *et al.*, 1999; Joyce *et al.*, 1991) being involved. Furthermore, biomechanical stresses imposed on the subchondral bone are increased in cartilage lesions (Chen *et al.*, 2011; Qiu *et al.*, 2003). As bone remodels in response to the increased load (Yokota *et al.*, 2011), this response may be inappropriate, possibly inducing subchondral bone plate migration and the formation of intralesional osteophytes.

Reviewer II: Do they have some suggestions for controlling subchondral bone changes in future repair techniques?

Authors: Regarding vascularisation processes as one possible aetiopathology, two interesting approaches to avoid subchondral bone overgrowth have been reported by Ernst Hunziker, the so-called structural barrier principle and the functional barrier principle. The structural barrier principle describes the implantation of a cell and blood vessel-excluding membrane (Millipore® or Goretex®), inserted at the interface between cartilage and bone compartments within osteochondral defects (Hunziker *et al.*, 2001). The functional barrier principle implies the addition of an anti-angiogenic factor (suramin) to the chondrogenic matrix implanted into chondral defects in order to prevent vascularisation within the cartilaginous compartment (Hunziker and Driesang, 2003). In agreement, Klinger *et al.* (2011) significantly inhibited terminal chondrocyte hypertrophy, the invasion of vessel structures, and excessive endochondral ossification by overexpression of chondromodulin-1 in cartilage defects in the knee joints of miniature pigs that were treated by microfracture. With regard to the enhanced biomechanical stresses acting on subchondral bone underlying cartilage lesions, it remains to be elucidated whether different postoperative weight-bearing regimes (Anderson and Smith, 2009) may delimit the upward migration of the subchondral bone plate or the formation of intralesional osteophytes. The authors have recently demonstrated that a bone anabolic pharmacological therapy with PTH [1-34] enhances the microarchitectural patterns of the repaired subchondral bone plate as well as of the subarticular spongiosa in a rabbit model of osteochondral defects (Orth *et al.*, 2013a).

Reviewer III: Can the authors give some of their ideas as to why some osteochondral defects (in animal models) give rise to bone plate overgrowth, or subchondral cysts, and others do not?
Authors: Interestingly, no hypothesis has yet been provided to explain the described pathological changes in the subchondral bone. Possible aetiopathologies of subchondral bone overgrowth include, but may not be limited to, impaired bone and articular cartilage regeneration, pathological vascularisation and angiogenesis, pathological structural consequences of altered biomechanical loading, hypertrophy of bone marrow-derived mesenchymal stem cells, and a disturbed mechanism of articular cartilage/subchondral bone crosstalk. Differences in the inter-individual intensity of their occurrence may explain in part why some osteochondral defects give rise to bone plate overgrowth and others do not. With regard to the formation of subchondral bone cysts, differences between animal species and humans have been described to account for the disparity in their development. In the sheep model, for example, there is a relatively high concentration of inflammatory cytokines (Benazzo *et al.*, 2008) and activated matrix metalloproteinase-2 (Miyamoto *et al.*, 2002) in the ovine synovial fluid when compared with the situation in humans. These factors, together with an elevated mean body temperature (38-40 °C) (Igono *et al.*, 1983; Recabarren *et al.*, 1987) compared to humans, may favour the development of cysts in the subarticular spongiosa in sheep. In addition, the different biomechanical

loading of the articular cartilage defect, based on differences in the range of motion and knee resting positions in various quadruped animal models compared to humans (Orth and Madry, 2013b; Pape and Madry, 2013), could also play a role. However, the definite reasons why some cartilage defects develop subchondral bone alterations while others do not (even in comparable animal models) will have to be further investigated in future studies.

Reviewer III: Concerning data in Table 2: do you think that the different frequency of bone plate overgrowth following microfracture reported by different orthopaedic surgeons could be related to surgical technique, i.e. different levels of aggressiveness in debridement of the lesion by each surgeon, or different surgical tools used to debride, prior to microfracture? Or, is it possible that the different clinical outcomes could be related to the different types of patients referred to the clinic (i.e., sports medicine *versus* more general population)?

Authors: Indeed, the different frequency of bone plate overgrowth following microfracture reported by different orthopaedic surgeons may be related to both the surgical technique and the heterogeneously distributed patient cohorts between the studies. With regard to the surgical technique, the work of Drobnic *et al.* (2010) has indeed demonstrated for the cartilage debridement step (prior to any marrow stimulation procedure or ACI) that the choice of surgical instrument critically affects the outcome with regard to completeness in cartilage removal and unintended injury of the subchondral bone plate. Likewise, aggressiveness in debridement or marrow stimulation would of course be another possible factor affecting the development of subchondral bone alterations. To date, no clinical study has yet evaluated the impact of patient age, degree of physical activity, etc. on the incidence of subchondral bone alterations during osteochondral repair. Although it has been shown that young patients with defects on the femoral condyles have the best prognostic factors for the microfracture treatment (Kreuz *et al.*, 2006), it remains unknown which demographic factors

favour the development of subchondral bone alterations. In conclusion, more high quality randomised controlled clinical trials will have to address these important questions in the future.

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