Autografts for spinal fusion: osteogenic potential of laminectomy bone chips and bone shavings collected via high speed drill

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Abstract In case of revision or minimal invasive spinal surgery, the amount of autograft possibly harvested from the lamina and the spinous processes is limited. Ekanayake and Shad (Acta Neurochir 152:651–653, 2010) suggest the application of bone shavings harvested via high speed burr additionally or instead, but so far no data regarding their osteogenic potential exist. Aim of the study was to compare the osteogenic potential of bone chips and high speed burr shavings, and to evaluate the applicability of bone shavings as an autograft for spinal fusion. Bone chips and shavings from 14 patients undergoing spinal decompression surgery were analyzed using in vitro tissue culture methods. Osteoblast emigration and proliferation, viability and mineralization were investigated and histological evaluation was performed. Bone chips from all patients showed successful osteoblast emigration after average 5.5 days. In contrast, only 57% of the corresponding bone shavings successfully demonstrated osteoblast emigration within an average time span of 14.8 days. Average osteoblast mobilisation was 1.25×10^6 cells per gram from bone chips and 1.73×10^5 cells per gram from the corresponding bone shavings. No difference was observed regarding cell viability, but population doubling times of bone chip cultures were significantly lower (50.5 vs. 121 h) and mineralization was observed in osteoblasts derived from bone chips only. Although some authors suggest the general applicability of laminectomy bone shavings as autografts for spinal fusion, autologous bone grafts obtained from laminectomy bone chips are superior in terms of cell delivery, cell proliferation and mineralization.

Keywords Spinal fusion · Bone graft · Osteoregeneration · Bone chips · High speed burr

Introduction

In order to achieve a solid spinal fusion, the PASS principles (Primary wound closure/Angiogenesis/Space/Stability) for predictable bone regeneration must be addressed [1]: primary wound closure is required to ensure undisturbed healing. Angiogenesis must take place to allow connection to the blood supply and attract undifferentiated mesenchymal stem cells to initiate bone regeneration. Space must be maintained or created in order to facilitate osteoblast ingrowth. Stability must be ensured to allow a blood clot formation maintaining growth factors relevant for cell differentiation at the fusion site. For all those reasons, stand alone bone grafts or bone grafts combined with fusion cages are used to promote bone healing and prevent pseudarthrosis formation.

Cancellous bone taken from the iliac crest is still considered a standard in spinal fusion, but is associated with complications in up to 20% [2]: complications include hematoma formation, neurological injuries and infection as well as acute and chronic pain. Studies report the incidence of chronic donor site pain for more than 2 years after surgery in 31% of the patients concerned [3]. Additional operating time and preparation is required, and bone harvesting may be insufficient [11, 12] Allograft bone is most frequently used as alternative, but lack of osteogenicity may decrease osteoinductance and increase infection rates [4]. Various types of synthetic bone grafts are rushing into

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the market, but their application is associated with additional costs and data on their effectiveness are still limited [5]. Autograft bone chips harvested from the laminae and spinous processes during decompression surgery are widely used, but their availability can be limited in case of revision surgery or infection [4].

Ekanayake and Shad [2] were the first to report the suitability of bone dust derived from drilling the posterior osteophyte as an autograft in anterior cervical fusion. Histological evaluation of those high speed burr shavings demonstrated that they are mainly composed of bone and blood products. The bone fraction contains viable osteoblasts without obvious microscopic damage caused by the burring process [6]. Bone shavings derived from drilling the lamina in order to get access to the intervertebral disc space might therefore represent an additional source of autograft bone for lumbar spinal fusion. This would also be advantageous for minimal invasive surgery, where less bone material can be acquired than via open techniques. Aim of the present study is an in vitro comparison of the osteogenic potential of laminectomy bone chips conventionally obtained by a kerrington rongeur and bone shavings harvested using a high speed burr equipped with a suction trap.

Materials and methods

Sample harvesting

Bone samples from 14 patients (13 female, 1 male) undergoing lumbar spinal surgery (decompression with/without fusion) were analyzed in the laboratory. Patients' ages were between 50 and 81 years (mean age 68 years). All patients showed a normal or slightly increased body weight (mean BMI 25.4) and no history of nicotine abuse or metabolic disease. Two patients had a record of cortisol treatment and three patients suffered from manifest osteoporosis.

The study was approved by the institutional ethics board. Bone samples were harvested from the laminae and the spinous processes during routine spinal decompression. Bone harvest from each patient was performed both as bone chips using a kerrington rongeur and via high speed drill (Ortho-TPS/Stryker) resulting in tiny bone shavings. Bone tissue was chilled using saline solution during the drilling process and collected using an in-line suction trap (B-collector/Intramed). After completion of cage and intervertebral space preparation, the remaining bone chips as well as the bone shavings were aseptically sent to the cell culture laboratory. Samples were placed separately in sterile falcon tubes pre-filled with Dulbecco's Modified Eagle's Medium (DMEM high glucose; Biomedica, Vienna/Austria) supplemented with 100 U/ml Penicillin

and 100 µg/ml Streptomycin (both Sigma–Aldrich, Vienna/Austria) for transportation. Bone tissue was stored at room temperature for a maximum of 24 h prior to tissue culture initiation.

Tissue culture

Bone samples were washed twice in Phosphate Buffered Saline (PBS, Invitrogen Carlsbad/CA, USA) to remove contaminating erythrocytes. After removal of a sample for histological evaluation, wet weight of the remaining tissue was assessed. Tissue harvested by rongeur and drill was then placed in separate 10 cm^2 tissue culture plates. DMEM supplemented with 10% fetal calf serum (FCS; Biomedica, Vienna/Austria), 2 mM L-Glutamine (Invitrogen, Carlsbad/CA, USA), 0.05 mg/ml Ascorbic Acid (Sigma–Aldrich, Vienna/Austria), 100 U/ml Penicillin and 100 µg/ml Streptomycin was used as a culture medium. Culture plates were checked daily for osteoblast emigration and culture viability. Culture medium was replaced twice a week.

Histological evaluation

Samples of bone tissue harvested either by kerrington rongeur or high speed drill were fixed in 4% formaldehyde (institutional pharmacy) for 48 h, followed by decalcification in Osteosoft® (VWR/Vienna, Austria) and paraffin embedding. 5 µm sections were prepared and stained Haemalaun/Eosin according to Romeis and Boeck [7]. Histological slides were analyzed using an Olympus IX-71 microscope.

Passage 1 osteoblast emigration cultures of both study groups were harvested via Trypsin–EDTA detachment as described below. Cells were seeded on four well-chambered slides (Sigma–Aldrich, Vienna/Austria) and cultured to subconfluency. Slides were then rinsed in PBS and fixed in methanol (VWR/Vienna, Austria) for 10 min at room temperature, followed by rinsing in distilled water and Alizarin red staining to detect Calcium deposition indicating mineralization. Alizarin red staining protocol was obtained from Histoweb [8].

Evaluation of osteoblast emigration

Osteoblast emigration from the source tissue was checked on a daily basis and the attachment of the first osteoblast to the culture dish was noted. As soon as all tissue samples of a study group demonstrated osteoblast emigration (which was referred to as a 100% emigration rate), the emigration rate of the corresponding bone sample was determined. Cells were then allowed to emigrate from the source tissue and allowed to multiply for 3 weeks in vitro. After that period, source tissue was removed and the osteoblasts were

harvested. To harvest the cells, cultures were rinsed in PBS followed by osteoblast detachment using 0.25% Trypsin–EDTA (Invitrogen/Carlsbad, CA, USA). Trypsin incubation was stopped by adding culture medium to a 1:1 ratio, and cell suspensions were washed twice in PBS prior to cell viability and cell count determination. Finally, cell yield per gram bone tissue was calculated.

Evaluation of population doubling times and cell viability

Cells harvested after a 3 weeks observation period were seeded into 75 cm^2 tissue culture flasks. After confluence was reached, cells were harvested and counted as previously described. Population doubling times were calculated and cell viability was determined using the trypan blue dye exclusion test.

Statistical evaluation

SPSS 14.0 was used for statistical evaluation. Groups were compared using a 2-tailed student's t test at a 0.05 level of significance.

Results

Histological evaluation of bone chips collected using the kerrington rongeur showed—as expected—intact tiny pieces of bone. Concentric lamellae surrounding the Haversian Channels were visible and osteoblasts could be observed surrounded by lacunae of extracellular matrix. Intact blood vessels were also visible within the samples (Fig. 1a). Slides obtained from bone shavings showed a different aspect: Bone tissue was disintegrated into tiny fragments, and although remnants of the interstitial lamellae were observed, osteoblasts were separated from their lacunae and located loosely between the lamellar fragments. Blood vessels were not observed (Fig. 1b).

Regarding osteoblast emigration, tissue harvested by the kerrington rongeur demonstrated reliable osteoblast release after average 5.6 days. Corresponding bone tissue obtained via high speed drill showed a high variation regarding osteoblast delivery: Although all rongeur samples from all patients—no matter whether obese, osteoporotic or after cortisol treatment—demonstrated successful osteoblast delivery to the culture dish, only 8 out of 14 corresponding samples (57%) harvested via high speed burr were able to do so ($p < 0.024$). The time span of osteoblast delivery—if any—was highly variable between 7 and 30 days (average time span 14.8 days; $p < 0.003$; Fig. 2).

After a 3 weeks culture period, average 1.25×10^6 osteoblasts could be obtained from the rongeur samples.
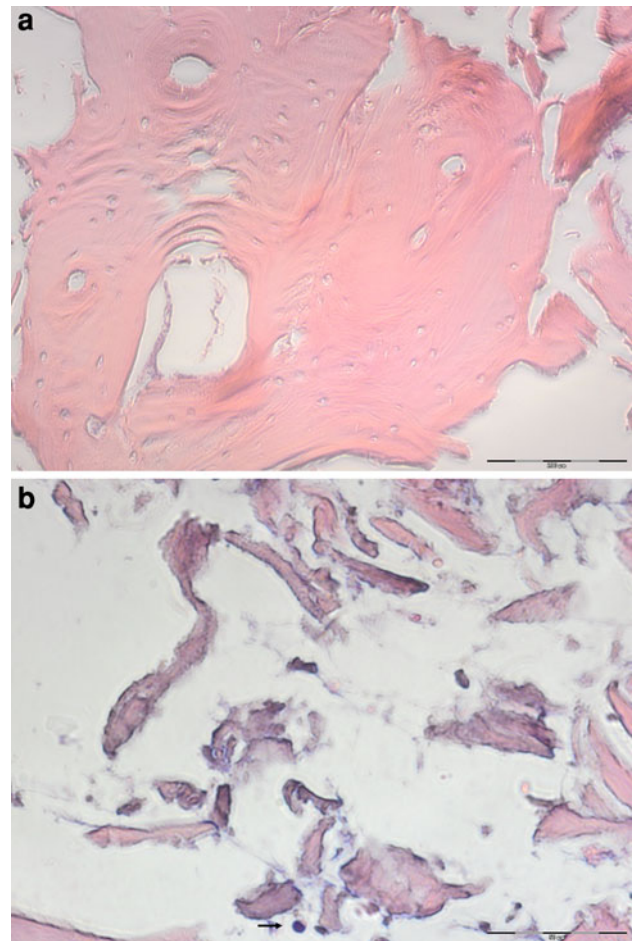

Fig. 1 a Laminectomy bone chips demonstrating intact lamellar bone structure, osteoblasts within their lacunae and blood vessel supply. **b** Bone shavings showing disrupted bone structure with single osteoblasts (*arrow*) deprived from their extracellular matrix. Blood vessels are not observed

Average cell yield obtained from the corresponding high speed burr samples was about 7× lower with an average of 1.73×10^5 osteoblasts per gram bone ($p < 0.01$, Fig. 3). Despite the highly different emigration time span and cell yield, viability in both study groups was equal at 98%.

Differences were also observed regarding population doubling times: osteoblasts emigrated from rongeur bone chips duplicated within 50.5 h while the corresponding bone shavings required 121 h for cell cycle completion ($p < 0.01$; Fig. 4). After 3 weeks of in vitro culture, positive Alizarin red staining indicating mineralization of monolayer tissue was only visible in cultures derived from rongeur bone chips (Fig. 5a, b).

Discussion

Although laminectomy bone shavings are used by spine surgeons to enhance fusion in cervical spine, only one
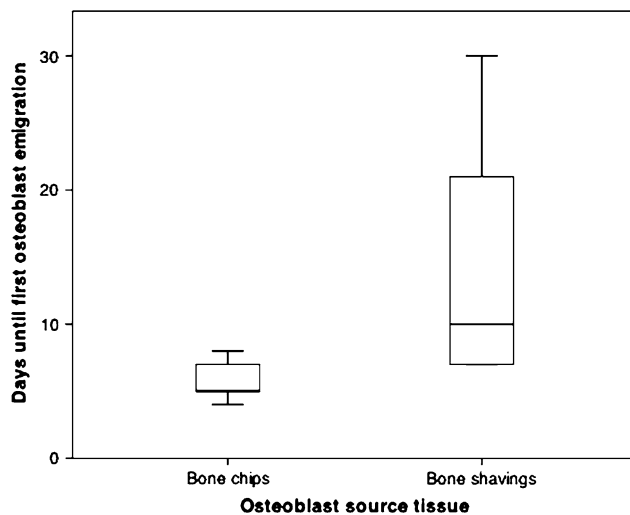
Fig. 2 Bone chips demonstrated successful osteoblast emigration after an average of 5.6 days. In corresponding bone shavings, successful osteoblast emigration was only observed in 57% of the samples and started after average 14.8 days ($p < 0.003$)
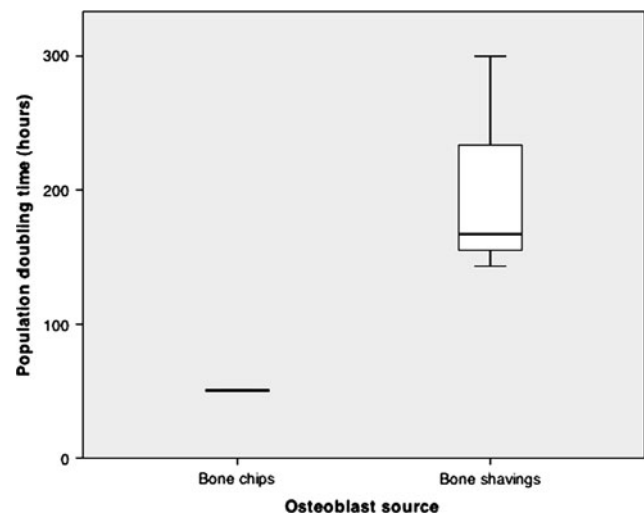

Fig. 4 Osteoblasts emigrated from rongeur bone chips duplicated within 50.5 h while the corresponding bone shavings required 121 h to complete a cell cycle ($p < 0.01$). PDT, population doubling time
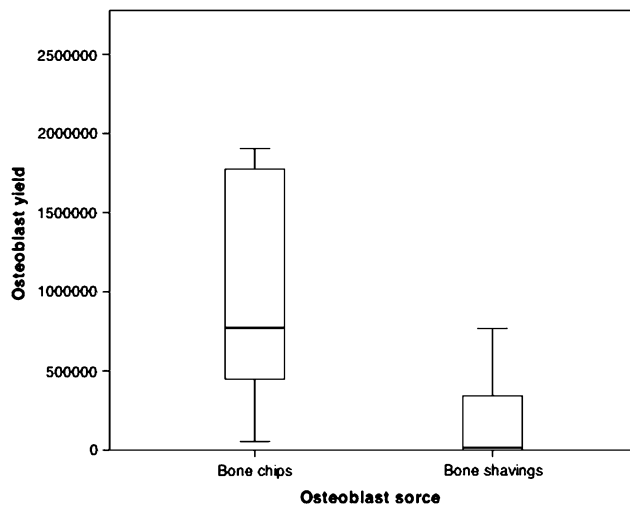

Fig. 3 After 3 weeks culture period, average 1.25×10^6 osteoblasts could be obtained from laminectomy bone chips. Average cell yield obtained from the corresponding bone shavings was about 7× lower with an average of 1.73×10^5 osteoblasts per gram ($p < 0.01$)

clinical paper is available investigating their suitability as an autograft [2]. Ekanayake and Shad reported the fitting of previously collected bone dust, together with 3–5 ml blood, into Solis interbody cages in eight patients. According to the authors, bone shavings provide a sufficient amount of bone even for a 2-level-discectomy, but no data regarding patient outcome or fusion success are provided.

Patel et al. [6] performed histological studies on laminectomy bone shavings collected during surgical decompression in 10 patients. They reported the resulting bone material to be viable without any obvious damage
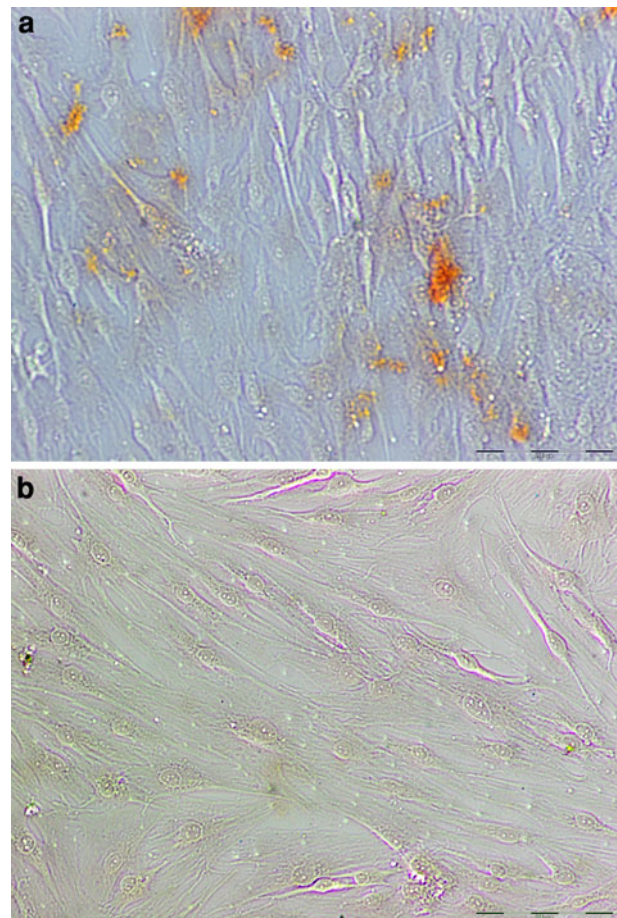

Fig. 5 a Osteoblast cultures derived from laminectomy bone chips demonstrate positive Alizarin Red staining indicating beginning mineralization after 3 weeks in vitro. **b** Alizarin Red staining remains negative in cultures derived from laminectomy bone shavings after a 3 weeks culture period

caused by the drilling process, but these findings should be regarded with care as evaluation was performed on the tissue in toto and no specific viability analysis was performed.

The presented study is the first investigating the osteogenic potential of high speed burr shavings in an in vitro setting, using bone chips from the same patient as control group. Our experiments revealed significant differences regarding the osteogenic potential of bone shavings and their corresponding bone chips: laminectomy bone chips showed a 100% osteoblast emigration rate after an average timespan of 5.6 days. In contrast, only 57% of the corresponding high speed burr shavings were able to release osteoblasts after an average timespan of 14.8 days. In vitro cultures of osteoblasts derived from bone shavings demonstrated significantly longer doubling times and did not show mineralization after 3 weeks in vitro.

Despite the assumption that high speed drills produce less heat than conventional drills and can be chilled down further by irrigation in saline solution [7], bone temperature raise during the shaving process might explain the poorer performance of osteoblast cultures derived from bone shavings. Damage to bone tissue due to heat shock is well known as the main cause of failure in osseointegration [9]. Exposing osteoblasts to a temperature of 45°C for 10 min results in a reversible disintegration of their actin filaments. Reversibility of cytoskeleton degradation is lost if the surrounding temperature is raised above 48°C [9].

As osteoblast viability in both study groups turned out equal, differences in osteogenic potential might also be the result of mechanical disruption rather than temperature stress. This consideration is supported by the histological analysis of high speed bone shavings demonstrating disintegrated osseous structures and osteoblasts deprived from their extracellular matrix. As the extracellular matrix is known to contain several osteopromoting factors [10], this might also explain the delay in osteoblast migration, doubling time and mineralization.

Although high speed burr shavings are capable of osteoblast release and proliferation, their in vitro results are inferior compared to the usage of conventional bone chips. The application of high speed burr shavings as an autograft for spinal fusion is basically possible, but might result in delayed fusion and poorer fusion rates compared to conventional bone chip harvesting.

Conflict of interest None.

References

1. Wang H, Boyapathi L (2006) PASS principles for predictable bone regeneration. Implant Dent 15(1):8–17
2. Ekanayake E, Shad A (2010) Use of the novel ANSPACH bone collector for bone autograft in anterior cervical discectomy and cage fusion. Acta Neurochir 152:651–653
3. Sasso RC, LeHuec JC, Shaffrey C. (2005) Iliac crest bone graft donor site pain after anterior lumbar interbody fusion: a prospective patient satisfaction outcome assessment. J spinal Dis Tech 18: S77–S778
4. Chien-Lung C, Chien-Lin L, Shih-Sheng S et al. (2006) Posterolateral lumbar spinal fusion with autogenous bone chips from laminectomy extended with OsteoSet®. J Chin Med Assoc 69(12):581–584
5. Agarwal R, Williams K, Umscheid CA et al. (2009) Osteoinductive bone graft substitutes for lumbar fusion: a systematic review. J. Neurosurg Spine (6):729–740
6. Patel V, Estes S, Naar E et al. (2009) Histologic evaluation of high speed burr shavings collected during spinal decompression surgery. Orthopedics 32(1):23
7. Romeis B, Boeck P (1989) Mikroskopische Technik. 17.Auflage. Urban and Schwarzenberg. Muenchen/Wien/Baltimore
8. Alizarin Red S Staining Protocol for Calcium. http://www.ihcworld.com/_protocols/special_stains/alizarin_red_s.htm. (Accessed 3 March 2011)
9. Li S, Chien S, Branemark PI (1999) Heat shock-induced necrosis and apoptosis in osteoblasts. J Orthop Res 17(6):891–899
10. Millett P, Allen M, Rushton N (1995) Synthetic function and regulation of osteoblasts: current knowledge and applications. McGill J Med 1:138–146
11. Banwart JC, Asher MA, Hassanein RS (1995) Iliac crest bone graft harvest site donor site morbidity. A statistical evaluation. Spine 20(9):1055–1060
12. Sawin PD, Traynelis VC, Menezes AH (1998) A comparative analysis of fusion rates and donor-site morbidity for autogeneic rib and iliac crest bone grafts in posterior cervical fusions. J Neurosurg 88(2):255–265