Quantitative Analysis of Growth Factors from a Second Filter Using the Reamer-Irrigator-Aspirator System: Description of a Novel Technique

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KEYWORDS

• Reamer-Irrigator-Aspirator (RIA) • Bone grafting
• Long bone nonunion • Growth factors • TCP filter

In the United States 7.9 million fractures occur annually. Between 5% and 10% of these fractures result in delayed or impaired healing. More than 1.5 million bone-grafting procedures are performed each year. Iliac crest bone graft has long been considered the gold standard for autogenous bone graft because of its osteoconductive, osteoinductive, and osteogenic properties[1]; however, it is associated with significant donor site morbidity and increased operative time.[2] The Reamer-Irrigator-Aspirator (RIA; Synthes, USA, Paoli, PA) was initially developed to potentially reduce the embolic load associated with reaming the femur for intramedullary nailing in trauma.[3] Subsequently it has been noted that the reamings are biologically active and may provide a source of autogenous bone similar to iliac crest in efficacy, but with fewer donor site complications.[4–10] The RIA system typically uses a single filter to capture the bony reaming debris from the RIA cutting head. Recently it has been noted that the irrigating fluid effluent may also contain significant amounts of growth factors and mesenchymal stem cells.[6,7]

The purpose of this paper is twofold. First, we report on our novel technique of using a second filter containing beta-tricalcium phosphate (TCP) as a graft extender while using the RIA system. Second, we sought to identify whether growth factors known to be present in the bony reaming debris and fluid effluent[6,7,9] are also present in the collections from the TCP filter.

MATERIALS AND METHODS

This study was approved by our Institutional Review Board (IRB). Informed consent was obtained from 16 patients who were prospectively enrolled between February 2007 and May 2007. Inclusion criteria included age older than 19 years and nonunion of a long bone requiring bone grafting; exclusion criterion was active infection at the time of the bone-grafting procedure. Patients

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could be enrolled if they had a history of prior infection, but no signs of active infection. There were eight males and eight females enrolled with an average age of 40 years (range 22 to 60 years). Seven (44%) of the 16 patients were smokers, 1 (6%) had diabetes, and 9 (56%) had an open fracture with their initial injury. All patients underwent bone harvest and grafting using the RIA system as well as iliac crest aspiration. Samples from the TCP filter were analyzed and compared with the patient's iliac crest bone marrow aspirate.

OPERATIVE RIA TECHNIQUE

Patients were placed supine on a radiolucent table. Bone was harvested using the RIA system through a trochanteric entry portal over a guide wire designed for this system. Access to the trochanteric portal was through a 25- to 30-mm incision that ended approximately 2 cm proximal to the tip of the greater trochanter. The technique is identical to trochanteric intramedullary nailing until the reamer is placed. A threaded wire was placed at the tip of the greater trochanter and drilled into the proximal femur. A cannulated opening reamer was then used to open the proximal femur. A ball-tipped guide wire was then inserted and passed into the distal femur. It is critical to make sure that the guide wire is centered distally. Two 750-μm sterile filters were attached to the suction tubing in series (**Fig. 1**). The reamer head diameter was determined using intraoperative fluoroscopy. The reamer was used in a single-pass fashion using a slow, deliberate advance/withdrawal maneuver. The first filter collected the bony reamings and the second filter contained either large (2.8–5.6 mm) or medium (1.4–2.8 mm)-sized TCP granules that were washed with aspiration fluid. For all subjects, three stratified (top, middle, and bottom) samples (**Fig. 2**) were taken from the TCP-containing second filter for quantitative analysis of various growth factors. The factors analyzed were vascular endothelial growth factor (VEGF), bone morphogenetic protein-2 (BMP-2), transforming growth factor-beta (TGF-beta), and total protein (TP). Each patient also had iliac crest bone marrow aspirate sent for quantification of these same growth factors. The entry portal was then plugged with a piece of tricalcium phosphate.

PROTEIN AND GROWTH FACTOR ANALYSIS

Samples for total protein analysis were homogenized and centrifuged. Total protein concentration in the supernatant was quantified using the BIO-RAD protein assay kit according to manufacturer protocol. VEGF, BMP-2, and TGF-beta concentration measurements were done using enzyme-linked immunosorbent assay (ELISA) methods from R&D Systems according to manufacturer protocol. Briefly, standards and samples were added to pre-coated plates and incubated. These were then washed with buffer and appropriate conjugates were added and incubated. After another washing these were incubated with the appropriate substrate. Stop solution was then added and the plate was read at the appropriate wavelength.

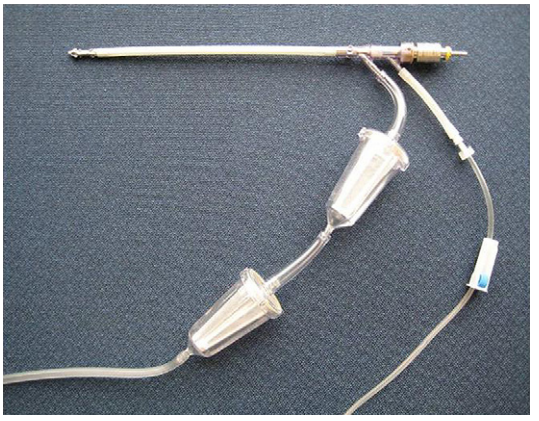

Fig. 1. RIA system reamer with attached irrigation and suction tubing. Filters are in series on suction tubing. Filter 1 captures bony reamings and filter 2 contains TCP granules.

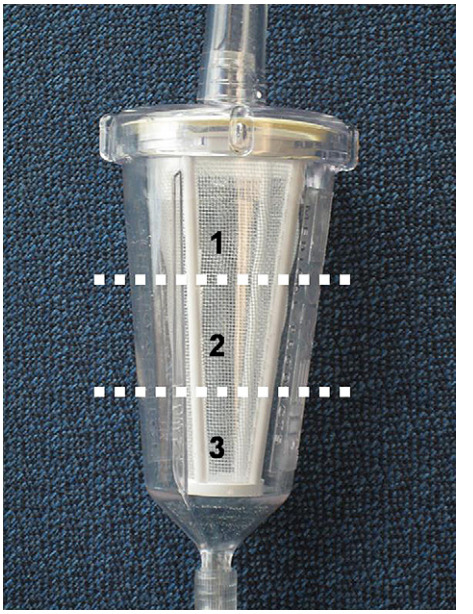

Fig. 2. Filter used in system. Samples were retrieved from the top (**1**), middle (**2**), and bottom (**3**).

STATISTICAL ANALYSIS

Stata 10 (StataCorp, College Station, TX) was used to analyze the data. Visual analysis of the data demonstrated that the data followed a relatively normal distribution, therefore a *t* test was used to compare the mean of the iliac crest bone aspirate with the mean of each sample location for each growth factor.

RESULTS

The concentration of VEGF, BMP-2, TGF-beta, and total protein in the TCP-containing second filter was determined by the ELISA method and compared with the concentration in iliac crest bone marrow aspirate. These data are presented in **Tables 1–4**. The mean concentration of VEGF in TCP (9.26 pg/mg) was significantly lower than that found in iliac crest aspirate (14.38 pg/mg), $P = .048$. BMP-2 was also found at a significantly lower concentration in TCP (10.41 pg/mg) compared with iliac crest (17.01 pg/mg), $P = .012$. TGF-beta was noted at more than double the concentration in TCP (58.58 pg/mg) compared with iliac crest (27.87 pg/mg), $P = .000$. Total protein concentration in TCP (27.68 mg/g) was nearly triple the concentration found in iliac crest aspirate (9.74 mg/g), $P = .000$. Among the three fractions within each TCP filter there did not seem to be any consistent relationship between concentration and location. Granule size did not affect BMP-2, TGF-beta, or VEGF levels collected in the TCP ($P = .753$, $P = .466$, $P = .509$ respectively). A mean of 40 mL of bone graft was obtained with a range of 18 to 55 mL.

DISCUSSION

The RIA is a new system that was initially developed to minimize the potentially harmful pulmonary effects of reaming during intramedullary nailing of femur fractures.[3] Subsequently it has been found to be an alternative method of autogenous bone graft harvest for reconstructive procedures[4,5,8,10] with fewer donor-site complications.[2,4,5,9] The bony reamings harvested using RIA contain osteogenic cells,[6,8] osteoinductive proteins,[7,8] and osteoconductive bony spicules and/or matrix.[8] Newer work suggests that in addition to the actual bony reamings, the irrigating fluid effluent may also contain growth factors and cells that could enhance the overall osteogenic potency of RIA graft. Schmidmaier and colleagues[7] recently demonstrated that both RIA bony reaming debris and the irrigation fluid contain rich amounts of growth factors important in bone formation. They quantified concentrations of platelet-derived growth factor (PDGF), BMP-2, fibroblast growth factors (FGF), VEGF, insulin-like growth factor (IGF-I), and TGF-beta and found a content comparable with that of iliac crest bone graft. Porter and colleagues[6] recently analyzed the cellular component as well as the protein component of RIA filtrate among five patients undergoing hip hemiarthroplasty. They found that the filtrate contains viable mesenchymal stem cells in addition to growth factors such as FGF-2, IGF-I, and TGF-beta1. They suggest that the true osteogenic potential of RIA filtrate may be associated with these musculoskeletal progenitor cells.

To our knowledge we are the first to describe using a second filter filled with TCP to enhance the collection process. This study is part of a larger

Table 1
Vascular endothelial growth factor, pg/mg

	Mean	SD	*P* Value
Iliac crest	14.38	11.95	Reference
TCP (top)	8.12	6.00	.031
TCP (middle)	8.46	11.56	.088
TCP (bottom)	11.19	10.07	.168
TCP (mean)	9.26	7.86	.048

Abbreviation: TCP, beta-tricalcium phosphate.

Table 2
Bone morphogenetic protein-2, pg/mg

	Mean	SD	*P* Value
Iliac crest	17.01	7.34	Reference
TCP (top)	11.73	8.83	.103
TCP (middle)	9.31	4.35	.002
TCP (bottom)	10.19	7.26	.014
TCP (mean)	10.41	5.49	.012

Abbreviation: TCP, beta-tricalcium phosphate.

Table 3
Transforming growth factor-beta, pg/mg

	Mean	SD	*P* Value
Iliac crest	27.87	5.93	Reference
TCP (top)	57.61	20.78	.000
TCP (middle)	67.47	32.43	.000
TCP (bottom)	50.63	19.19	.001
TCP (mean)	58.58	16.35	~.000

Abbreviation: TCP, beta-tricalcium phosphate.

Table 4
Total protein, mg/g

	Mean	SD	*P* Value
Iliac crest	9.735	1.97	Reference
TCP (top)	32.98	19.82	.003
TCP (middle)	25.31	10.78	.000
TCP (bottom)	24.74	7.37	.000
TCP (mean)	27.679	8.41	~.000

Abbreviation: TCP, beta-tricalcium phosphate.

ongoing investigation into the use of RIA for autologous bone graft harvest. Our initial thought process was to have the TCP serve as an osteoconductive graft extender and to increase the overall volume of the graft harvest. We harvested a mean of 40 mL of graft and then added the TCP to that as a graft extender. This is in keeping with previous reports on graft volume.[4,5,8] We intentionally harvested no more than was necessary, and in all cases we had a sufficient quantity of bone graft. In light of the aforementioned recent work on growth factors in the bony reaming and fluid effluent,[6,7] we also investigated whether these same growth factors are present in the TCP filter. We showed that they are indeed present in substantial amounts. TGF-beta and total protein levels in TCP were higher than in iliac crest aspirate. Although VEGF and BMP-2 levels were lower in TCP compared with iliac crest, significant concentrations were nonetheless present. As part of a larger ongoing study we compared the concentrations of these growth factors in TCP with that in RIA bony reamings and found no statistically significant difference.[9] We did not expect the TCP filter to have equal concentrations of growth factors with the actual bone graft harvested. Neither the size of the TCP granules nor the location within the filter appears to impact growth factor concentrations.

In conclusion, our data indicate that a second filter filled with TCP is not simply a passive osteoconductive graft extender. Our findings imply that significant amounts of additional growth factors can be harvested using a second filter filled with TCP. We believe this is an easy and reproducible modification of the standard RIA technique that could enhance the osteogenic properties of RIA bone graft. Future work could focus on quantifying the amount of viable mesenchymal stem cells in the second filter filled with TCP. Other areas of research could investigate the impact of using different osteoconductive materials in the second filter. A second filter attached in series with the standard RIA filtration system yields TCP with substantial concentrations of bioactive proteins that are equal to those seen in the bone graft that is harvested in the first filter.

REFERENCES

1. Goldberg VM, Akhavan S. Bone grafts and bone graft substitutes. In: Friedlaender GE, Mankin HJ, Goldberg VM, editors. AAOS monograph series 32. 1st edition. Rosemont (IL): American Academy of Orthopaedic Surgeons; 2006. p. 1–8.
2. Ebraheim NA, Elgafy H, Xu R. Bone-graft harvesting from iliac and fibular donor sites: techniques and complications. J Am Acad Orthop Surg 2001;9: 210–8.
3. Muller CA, Green J, Sudkamp NP. Physical and technical aspects of intramedullary reaming. Injury 2006;37:S39–49.
4. Belthur MV, Conway JD, Jindal G, et al. Bone graft harvest using new intramedullary system. Clin Orthop Relat Res 2008;466(12):2973–80.
5. Newman JT, Stahel PF, Smith WR, et al. A new minimally invasive technique for large volume bone graft harvest for treatment of fracture nonunions. Orthopedics 2008;31(3):257–61.
6. Porter RM, Liu F, Pilapil C, et al. Osteogenic potential of Reaming Irrigator Aspirator (RIA) aspirate collected from patients undergoing hip arthroplasty. J Orthop Res 2009;27(1):42–9.
7. Schmidmaier G, Herrmann S, Green J, et al. Quantitative assessment of growth factors in reaming aspirate, iliac crest, and platelet preparation. Bone 2006; 39:1156–63.
8. Stafford PR, Norris B. Reamer-irrigator-aspirator as a bone graft harvester. Tech Foot Ankle Surg 2007; 6(2):100–7.
9. Stannard JP, Volgas DA, Stewart RL, et al. Autograft bone graft harvest using an intramedullary reamer. Poster presented at: Orthopaedic Trauma Association 23rd Annual Meeting. Boston (MA), October 17–20, 2007.
10. Weatherby B, Norris BL, Nowotarski P, et al. Reamer irrigator aspirator (RIA) bone graft harvesting in nonunion and segmental defect repair. Poster presented at: American Academy of Orthopaedic Surgeons Annual Meeting. San Diego (CA), February 14–18, 2007.