# A RANDOMIZED, SINGLE BLIND, COMPARATIVE STUDY ON THE EFFECTS OF TOPICAL CALCIUM GLYCEROPHOSPHATE ON SURGICAL WOUND HEALING AND RESIDUAL SCARRING IN BILATERAL TOTAL KNEE ARTHROPLASTY PATIENTS

Alvin Ong, M.D.[1], Fabio Orozco, M.D.[1], Emran S. Sheikh, M.D.[1],
Craig Anmuth, D.O.[2], Abraham Alfaro, Ph.D., D.O.[2], Richard Kathrins, Ph.D.[2]
Gary Lee Grove, Ph.D.[3], Charles Zerweck, Ph.D.[3],
Anne Marie Madden, CRC[1], Robert Raspa PA-C[1]
Margaret T. Weis, Ph.D.[4*]

[1]Rothman Institute
Philadelphia PA 19107-4216

[2]Bacharach Institute
Pomona, NJ 08240

[3]cyberDERM Clinical Studies
Broomall, PA 19008

[4]Department of Biomedical Sciences
School of Pharmacy
Texas Tech University Health Sciences Center
Amarillo, TX 79106

Address correspondence to:
margaret.weis@ttuhsc.edu

Study Sponsor: AkPharma Inc.
6840 Old Egg Harbor Road
P O Box 111
Pleasantville, NJ 08232

ABSTRACT

Rapid wound healing is associated with improved surgical outcomes. While therapies to increase wound healing have been pursued in both laboratory and clinical studies, current standards of care include no pharmacologic agents that improve wound healing. The present study tested the hypothesis that topically applied calcium glycerophosphate would improve surgical wound healing following bilateral knee replacement. Twenty otherwise healthy subjects scheduled for bilateral total knee arthroplasty completed this study; for each patient, one knee was randomly assigned to the treatment group, while the contralateral knee was designated control. The subjects were instructed to apply a preparation of 10% calcium glycerophosphate in an aqueous lotion to the treated knee once daily for 42 days, starting at the 3rd post-operative day. Functional sealing and cosmetic appearance of the incision were evaluated from photographs by expert graders, who scored the intensity and extent of erythema along the incision and over the entire knee, the appearance of visible edema along the incision and over the knee, and the global impression of wound healing. Both the area and intensity of erythema along the incision were significantly reduced in the treated *vs.* untreated knee. Treatment significantly reduced edema, both along the incision and globally, across the entire knee. The differences were most marked at the $7^{th}$ post-operative day and diminished with time. No adverse effects were observed for any patient, in either treated or untreated knees. These data demonstrate that post-operative application of 10% calcium glycerophosphate improves surgical wound healing following total knee arthroplasty.

INTRODUCTION:

Rapid wound healing is associated with improved outcomes following orthopedic surgeries, including reduced incidence of surgical site infection (SSI), reduced scarring and improved range of motion. Therapies to increase the speed of wound healing have been pursued in both laboratory and clinical studies, yet current standards of care include no agents, topical or systemic, that increase the rate of post-operative wound healing and/or diminish long term scarring.

Slow wound healing is correlated with SSI [1] and significantly increased mortality risk [2,3]. Case record analysis suggests that total knee arthroplasty (TKA) has the highest rate of SSI of five common orthopedic procedures [4]. Infection is associated with athrofibrosis and consequent limited range of motion [5].

Surface scarring is associated with pain on movement [6,7] and is a significant factor associated with a patient's perceived ability to kneel following TKA [8]. Cosmetic considerations [9] aside, hypertrophic scarring and keloid formation are factors restricting range of motion following surgery [10], and have a negative impact on quality of life.

There is a significant body of literature showing that wound healing is a calcium dependent process [11]. *In vitro*, calcium stimulates keratinocyte differentiation [12], by stimulating sphingosine kinase [13-19] and protein kinases (Filvaroff *et al*, 1990[20-24]. These activate several transcription factors [25], stimulating angiogenesis and upregulating expression of cell-cell adhesion proteins [26]. Ultimately, these processes recruit tissue fibroblasts to repair, stimulate endothelial progenitor cells to vascularize and stimulate keratinocytes to re-epithelialize the wound.

The study reported here tested the hypothesis that topical application of calcium, in the form of calcium glycerophosphate (CGP), would speed wound healing. It is obvious that CGP can supply calcium to stimulate kinases. Less obviously, glycerophosphate is a phosphatase inhibitor [27,28]. The activity of the kinases is limited by phosphatase-mediated removal of phosphate groups from protein or lipid targets. Thus, we speculated that, by simultaneously

stimulating kinases and inhibiting phosphatases, CGP would speed surgical wound healing, and perhaps improve long-term cosmetic effects.

METHODS:

*Treatment Protocol:* Prior to initiation of the study, the proposed protocol, informed consent form and product information were approved by the Bacharach Institute for Rehabilitation Institutional Review Board. The protocol is registered as study NCT01264588 with www.ClinTrials.gov. The treatment schedule is detailed in Table 1.

*Subjects:* The CONSORT flowchart for patient eligibility, enrollment and retention is shown in Figure 1. Subjects meeting the inclusion/exclusion criteria (Table 2) were enrolled sequentially from a pool of candidates scheduled for bilateral knee replacement surgery. One week prior to surgery (visit 1), the study obligations and risks were explained and informed consent was obtained from each subject. Subjects were assigned by 1:1 randomization to apply the study product formulation topically once daily to either the left or right knee incision as assigned. The contralateral knee incision served as the control group. Baseline assessments were made at visit 2 (3rd post-operative day), prior to treatment.

*Randomization:* The sponsor prepared blinded treatment kits containing 42 pre-measured doses of the CGP preparation, application instructions, a treatment diary, and the subject's randomization assignment - RIGHT or LEFT. The blinded kits were dispensed by the clinical research coordinator in numerical sequence to subjects, who were also in numerical sequence. Kits were prepared in blocks of 4, each block comprising two designated LEFT, and two designated RIGHT. Until the kit box was opened by the clinical research coordinator in the patient's presence, the instructions as to leg assignment could not be read. Thus, the sponsor did not know which patient would receive which kit, and the surgeon did not know whether a particular kit had been designated RIGHT or LEFT.

*Calcium glycerophosphate:* CGP was prepared as a 10 % suspension (by weight) in an aqueous lotion base consisting of DL lactic acid (1.95%), cellulose gum (2.5%), glycerin (1%), methylparaben (0.2%), and water (84.35%). The study sponsor prepared and supplied all treatment kits.

*Methods:* Institutional wound care guidelines alone (CONTROL) were the standard of care against which CGP treatment was measured. That standard was to clean the wound site with normal saline daily. If so ordered, once the product was dry, both wounds could be covered with a Combine ABD pad held in place with TubiGrip™. The use of povidone-iodine (Betadyne®) on either wound was specifically avoided. Guidelines for TKA were modified to include application of topical CGP (ACTIVE) to the assigned knee. There were no standard alternatives.

At visit 2, each subject was provided with a treatment kit as described above. The subjects were instructed to apply the CGP preparation once daily to the experimental knee only, then to record the date and time of application on the kit diary form. The importance of proper application methods and frequency of application were explained, emphasizing that: 1) no products other than those provided were to be applied; 2) neither incision was to be tampered with in any way during the study period; and 3) contact between the treatment and control wound sites was to be avoided. Treatment continued daily for 42 days (6 weeks). Subjects were followed for an additional 46 weeks for a total study duration of 12 months. Patient compliance was verified at each study visit by examining the kits for unused medication.

*Wound Evaluation:* Digital photographs as objective measurements were made as per schedule (Table 1) by the clinical research coordinator in charge of the study, using a camera supplied by the sponsor. The photographs were coded with the patient number and did not indicate which was the treated knee. An orthopedic surgeon and a plastic surgery clinical provider, evaluated the differences between left and right knees, both by direct examination of the patient and from the photographs. In addition, two expert skin graders evaluated the differences between left and right knees from the photographs only. The evaluators (clinical providers and expert graders alike) were blinded as to which knee was treated.

Functional sealing and cosmetic appearance of the wound were measured by grading the intensity and extent of erythema along the incision and over the entire knee, the appearance of visible edema along the incision and over the knee, and the global impression of wound healing. All grading was recorded as left knee vs. right knee for each patient. The side perceived as worse was assigned a score of 0, while the side perceived as "better" was assigned a value of 1 (smallest difference) to 4 (greatest difference).

*Pain Evaluation:* Subjects were asked to rate their pain at the site of incision for each knee, using a scale of 0 (no pain) to 10 (excruciating pain). The pain evaluations were recorded on a pain diary sheet provided with the treatment kit.

*Range of Motion:* Range of motion (both flexion and extension) was evaluated by standard goniometric methods, as per the schedule shown in Table 1.

*Statistics:* Results were tabulated and analyzed by two-way analysis of variance using the Prism 5.0b software package. Assuming an independent two-sample t-test, our sample size of 20 subjects will provide at least 80% power to detect a medium to large effect size for a two-sided test of significance at a critical level of $p = 0.05$. All tests were against a two-sided alternative. Dropouts in this trial were sequentially replaced to maintain the power of the study.

RESULTS:

There were no surgical site infections in any patient, in either the treated or untreated knee.

Of the 40 patients assessed for eligibility, 20 completed the study. The first 15 patients enrolled were supplied product in a squeeze bottle. Compliance was evaluated at visit 5 by weighing the unused product. It was determined that 11 of these 15 patients were non-compliant. The study was placed on hold while the product packaging was changed to unit-dose format; subsequent patients were supplied only with unit dose product. There was 100% compliance with the new dispensing format. None of the photographs of the dropped patients were evaluated at any time for differences between treated and untreated knees. The final patient population is described in Table 3.

Wound healing and scar formation were photographically documented, as described in Methods. The results of a single representative patient at post-operative days 14 and 70 (corresponding to visits 4 and 6) are shown in Figure 2. By post-operative day 14 treatment had been applied for 10 days and by post-operative day 70 the 6 weeks of treatment had been completed. At post-operative day 14, there was a marked difference between the treated and untreated knees. While the difference had diminished by the 70$^{th}$ post-operative day, there is still a discernable difference between treated and untreated knees for this patient.

The expert graders' scores for all patients for erythema, edema and global impression of wound healing are shown in figures 3 through 5. As shown in figure 3, panels A and C, both the area and intensity of erythema along the incision were significantly reduced in the treated *vs.* untreated knee (overall probability = 0.003). The difference was greatest at visit 3 (7$^{th}$ post-operative day, 4$^{th}$ treatment day) and diminished with time. By the 8$^{th}$ visit (6 months post-operative), the scar appearances of the treated and untreated knees were no longer distinguishable. While treatment significantly reduced the area (Figure 2, panel B; overall p = 0.0019) and intensity (Figure 2, Panel D; overall p=0.003) of erythema over the entire knee, the effect was less visually obvious than that observed directly along the incision site.

The treated knees had significantly less edema, both along the incision (Figure 4, panel A; overall $p<0.0001$) and globally, across the entire knee (Figure 4, panel B; overall $p = 0.002$). As with erythema, the effect was greatest at visit 3, and diminished with time.

The global impression of wound healing was markedly and significantly (overall $p<0.0001$) better in the treated *vs.* the untreated knee (Figure 5). As anticipated, the effect diminished with time. At visits 3 through 9 the treated knees scored consistently higher than the untreated. However, at visit 9 the difference between treated and untreated appeared to be increasing, rather than decreasing.

Patients were asked to evaluate pain at the site of incision using a scale of 0 (no pain) to 10 (extreme pain). Overall, the reported pain levels were low. The mean pre-treatment pain score (3rd post-operative day) was $1.78 \pm 0.64$ for the treatment-designated *vs.* $1.75 \pm 0.63$ for the untreated knees (not significant). By visit 3 ($7^{th}$ post-operative day; $4^{th}$ treatment day), pain at the site of the incision had declined to $0.88 \pm 0.35$ in the treated *vs.* $0.85 \pm 0.40$ in the untreated knees (not significant). At no time was there a difference in the pain scores between the treated and untreated knees. Overall, the mean pain score declined with time, so that by visit 9 no patient reported pain associated with the incision in either knee.

Both flexion and extension were evaluated at the 3rd post-operative day (Visit 2, before initiation of CGP treatment), the $7^{th}$ post-operative day (Visit 3, after 4 days of CGP treatment), at the $6^{th}$ postoperative week (Visit 5, after 39 days of CGP treatment) and at 6 months and 1 year post-operatively. At visit 2, before treatment, the mean flexion was $66 \pm 3.3$ degrees for the knees assigned to the treatment group and $71 \pm 2.4$ degrees for the contralateral knees. These values were significantly different from each other ($p = 0.046$). As shown in figure 6, panel A, by 6 weeks, the treated knees had gained $40 \pm 3.5$ degrees of flexion, as compared to $34 \pm 3.2$ degrees for the contralateral knees ($p = 0.016$). By 6 months, there was no difference in flexion between treated and untreated knees.

At baseline, the mean extension was $-4.6 \pm 1.5$ degrees for the knees assigned to the treatment group, and $-4.4 \pm 1.6$ degrees for the contralateral knee (not significant). By 6 weeks,

the mean extension of the treated knee had improved by 4.4 ± 1.6 degrees as compared to 3.4 ± 1.8 degrees for the untreated knee (Figure 6, panel B). The probability that this difference occurred by chance was very small, and approached significance ($p = 0.07$). At 6 months and one year, the extension of all knees, treated or untreated, was zero.

DISCUSSION:

The objective of this study was to evaluate efficacy of topical calcium glycerophosphate (CGP) in the overall healing and appearance of surgical incisions. The following endpoints were sequentially evaluated for the first postoperative year: 1) improvement of surgical wound appearance; 2) reduction of visible erythema/-inflammation; 3) scar minimization or prevention; 4) patient-reported pain and scar sensitivity; and 5) range of motion. The results of this study demonstrate that topical CGP application significantly speeds wound healing in total knee arthroplasty. The difference was particularly marked at visit 3 ($7^{th}$ post-operative day) where better wound appearance was illustrated by reduced appearance of erythema and edema, suggesting reduced inflammation. While the differences between treated and untreated knees tended to diminish with time, at all time points the global impression of wound healing was discernibly better in the treated knee. Indeed, the differences between treated and untreated may have been increasing by the end of the first post-operative year.

At no time post-operatively did CGP treatment affect pain associated with the incision. However, patients did not report high levels of incision-associated pain for either knee. In part, perhaps, the incision-associated pain was low due to concurrent post-operative analgesia. In any case, CGP treatment did not increase scar-associated pain.

The data suggest that CGP treatment may be associated with earlier gain in range of motion in the immediate 6-week postoperative period. The treated knees gained flexion earlier than the untreated knees, suggesting that the reduced erythema/edema observed in the treated *vs.* untreated knees facilitated recovery of range of motion. However, this result must be interpreted with caution. The knees assigned to the treatment group started with a degree of flexion that was significantly worse than the contralateral knees. The gain in flexion may simply be an artifact attributable to the initial inequality. However, at the very least, the data show that CGP treatment did not impair recovery of range of motion.

The usual confounding factors of an arthroplasty wound healing study, *e.g.*, different surgical techniques, different implant devices and co-morbidities such as diabetes were absent

from these studies. Limiting the study to otherwise healthy patients having bilateral TKA has narrowed the number of variables, and allows us to conclude with a high degree of certainty that the differences observed between the treated and untreated knees are most likely the result of CGP therapy.

While eliminating co-morbidities such as diabetes does remove an important confounding factor, it also limits the interpretation of the study. That is, the data demonstrate a treatment effect in otherwise healthy patients. Arguably, the probability of observing increased speed of wound healing is lowest in this patient population. The effect of CGP treatment in patients suffering from chronic diseases is and remains unknown. The large treatment improvement observed in the present study suggests that even greater differences might be observed in the population with co-morbidities, such as diabetes, that impair wound healing. Although it is well beyond the scope of this study, a future study of CGP in these patients is clearly warranted.

Therapies to improve wound healing have been pursued in both laboratory and clinical studies. Although rapid wound healing is associated with improved surgical outcomes, current standards of care include no agents, topical or systemic, that speed post-operative wound healing. A number of studies have been directed to improving wound healing, particularly to investigation of platelet-derived growth factors or surface laser treatment. The results have not been encouraging.

Autologous platelet gels have been extensively explored as a means to speed wound healing in general [29] and TKA in particular [30-32]. The effects have been, at best, marginal, possibly due to the method of platelet preparation [33]. Alternatively, as platelet activation occurs during normal healing, applying additional platelet factors may not greatly stimulate a near-maximally stimulated system.

Although laser treatment is effective in surgical scar revision [10], studies of the efficacy of laser treatment to promote surgical wound healing yielded inconsistent results [34].

Platelet derived factors, laser therapy and CGP may act through a group of common effectors. Platelet lysates likely activate protein kinases [35,36], increasing fibroblast migration to the wound [37]. CGP certainly supplies free calcium, activating both platelets and kinases. Laser treatment, by generating heat, appears to activate a pathway that ends with the inhibition of phosphatases [38-41], and glycerophosphate is a general phosphatase inhibitor [27,28]

But proper preparation of autologous platelet gels is difficult, expensive, and exacting [33]. Successful laser treatment is expensive, and requires multiple treatments [40].

In contrast, CGP is inexpensive (less than $100 per knee for the 6-week course of therapy) and easy to apply. The present study shows that CGP promotes surgical wound healing in otherwise healthy subjects undergoing total knee arthroplasty, and suggests that it may improve the long term cosmetic appearance of the surgical scar. These properties suggest that CGP merits further investigation in a broader patient population, as well as for other surgical procedures.

REFERENCES:

1. Olsen MA, Nepple JJ, Riew D, et al. Risk factors for surgical site infection following orthopedic spinal operations. J Bone Joint Surg. 2008;90(1):62-69.
2. Lee J, Singletary R, Schmader K, et al. Surgical site infection in the elderly following orthopaedic surgery. Risk factors and outcomes. J Bone Joint Surg. 2006;88(6):1705-1712.
3. Merrer J, Girou E, Lortat-Jacob A, et al. Surgical infection after surgery to repair femoral neck fracture: a French multicenter retrospective study. Infect Control Hosp Epidemio. 2007;28(10):1169-1174.
4. Kandono Y, Yasunaga H, Horiguchi H, et al. Statistics for orthopedic surgery 2006-2007: data from the Japanese Diagnosis Procedure Combination database. J Orthop Sci. 2010;15(2):162-170.
5. Ries MD, Badalamente M. Arthrofibrosis after total knee arthroplasty. Clin Orthop Relat Res. 2000;380:177-183.
6. Sundaram RO, Ramakrishnan M, Harvey RA, Parkinson RW. Comparison of scars and resulting hypoaesthesia between the medial parapatellar and midline skin incisions in total knee arthroplasty. The Knee 2007;14(5):375-378.
7. Palmer SH, Servant CT, Maguire J, et al. Ability to kneel after total knee replacement. J Bone Joint Surg [Br]. 2002;84(2):220-222.
8. Schai PA, Gibbon AJ, Scott RD. Kneeling ability after total knee arthroplasty. Perception and reality. Clin Orthop Relat Res. 1999;367:195-200.
9. Moran M, Khan A, Sochart DH, Andrew G. Evaluation of patient concerns before total knee and hip arthroplasty. J Arthroplasty. 2003;18(4):442-445.
10. Alster TS, West TB. Treatment of scars: a review. Ann Plast Surg. 1997;39(4):418-432.
11. Lansdown AB. Calcium: a potential central regulator in wound healing in the skin. Wound Rep Reg. 2002;10(5):271-285.
12. Yuspa SH, Hennings H, Tucker RW, et al. Signal transduction for proliferation and differentiation in keratinocytes. Ann N Y Acad Sci. 1988;548:191-196.

13. Punnonen K, Denning M, Lee E, et al. Keratinocyte differentiation is associated with changes in the expression and regulation of phospholipase C isoenzymes. J Invest Dermatol. 1993;101(5):719-726.
14. Yatomi Y, Ruan F, Hakomori S, Igarashi Y. Sphingosine-1-phosphate: a platelet-activating sphingolipid released from agonist-stimulated human platelets. Blood. 1995;86(1):193-202.
15. English D, Garcia JG, Brindley DN. Platelet-released phospholipids link haemostasis and angiogenesis. Cardiovasc Res. 2001;49(3):588-599.
16. Santini MP, Talora C, Seki T, et al. Cross talk among calcineurin, Sp1/Sp3, and NFAT in control of p21(WAF1/CIP1) expression in keratinocyte differentiation. Proc Natl Acad Sci USA. 2001 Aug 14;98(17):9575-9580.
17. Berdyshev EV, Gorshkova IA, Usatyuk P, et al. De novo biosynthesis of dihydrosphingosine-1-phosphate by sphingosine kinase 1 in mammalian cells. Cell Signal. 2006;18(10):1779-1792.
18. Serriere-Lanneau V, Teixeira-Clerc F, Li L, et al. The sphingosine 1-phosphate receptor S1P2 triggers hepatic wound healing. FASEB J. 2007;21(9):2005-2013.
19. Yu H, Okada T, Kobayashi M, et al. Roles of extracellular and intracellular sphingosine 1-phosphate in cell migration. Genes Cells. 2009;14(5):597-605.
20. Filvaroff E, Stern DF, Dotto GP. Tyrosine phosphorylation is an early and specific event involved in primary keratinocyte differentiation. Mol Cell Biol. 1990;10(3):1164-1173.
21. Filvaroff E, Calautti E, Reiss M, Dotto GP. Functional evidence for an extracellular calcium receptor mechanism triggering tyrosine kinase activation associated with mouse keratinocyte differentiation. J Biol Chem. 1994;269(34):21735-21740.
22. Denning MF, Dlugosz AA, Williams EK, et al. Specific protein kinase C isozymes mediate the induction of keratinocyte differentiation markers by calcium. Cell Growth Differ. 1995;6(2):149-157.

23. Adhikary G, Chew YC, Reece EA, Eckert RL. PKC-delta and -eta, MEKK-1, MEK-6, MEK-3, and p38-delta are essential mediators of the response of normal human epidermal keratinocytes to differentiating agents. J Invest Dermatol. 2010;130(8):2017-2030.
24. Jadali A, Ghazizadeh S. Protein kinase D is implicated in the reversible commitment to differentiation in primary cultures of mouse keratinocytes. J Biol Chem. 2010 23;285(30):23387-23397.
25. Medema JP, de Vries-Smits AM, Bos JL. Calcium induces tyrosine phosphorylation of a novel p120GAP-associated protein of 65 kDa. Oncogene. 1995;11(4):757-762.
26. Calautti E, Cabodi S, Stein PL, et al. Tyrosine phosphorylation and src family kinases control keratinocyte cell-cell adhesion. J Cell Biol. 1998;141(6):1449-1465.
27. Novak-Hofer I, Thomas G. Epidermal growth factor-mediated activation of an S6 kinase in Swiss mouse 3T3 cells. J Biol Chem. 1985;260(18):10314-10319.
28. Ballou LM, Jenö P, Thomas G. Protein phosphatase 2A inactivates the mitogen-stimulated S6 kinase from Swiss mouse 3T3 cells. J Biol Chem. 1988;263(3):1188-1194.
29. Grotendorst GR, Martin GR, Pencev D, et al. Stimulation of granulation tissue formation by platelet-derived growth factor in normal and diabetic rats. J Clin Invest. 1985;76(6):2323-2329.
30. Gardner MJ, Demetrakopoulos D, Klepchick PR, Mooar PA. The efficacy of autologous platelet gel in pain control and blood loss in total knee arthroplasty. An analysis of the haemoglobin, narcotic requirement and range of motion. Int Orthop. 2007;31(3):309-313.
31. Peerbooms JC, deWolf GS, Colaris JW, et al. No positive effect of autologous platelet gel after total knee arthroplasty: A double-blind randomized controlled trial: 102 patients with a 3-month follow-up. Acta Orthopaedica. 2009;80(5):557-562.
32. Horstmann WG, Slappendel R, van Hellemondt GG, et al. Autologous platelet gel in total knee arthroplasty: a prospective randomized study. Knee Surg Sports Traumatol Arthrosc. 2011;19(1):115-121.

33. Gipponi M, Reboa G, Testa T, et al. Tension-free Primary Closure with Autologous Platelet Gel versus Vivostat- for the Definitive Treatment of Chronic Sacrococcygeal Pilonidal Disease. In Vivo. 2010;24(4):583-589.
34. Posten W, Wrone DA, Dover JS, et al. Low-level laser therapy for wound healing: mechanism and efficacy. Dermatol Surg. 2005;31(3):334-340.
35. Ranzato E, Mazzucco L, Patrone M, Burlando B. Platelet lysate promotes in vitro wound scratch closure of human dermal fibroblasts: different roles of cell calcium, P38, ERK and PI3K/AKT. J Cell Mol Med. 2009;13(8B):2030-2038.
36. Ranzato E, Boccafoschi F, Mazzucco L, et al. Role of ERK1/2 in platelet lysate-driven endothelial cell repair. J Cell Biochem. 2010;110(3):783-793.
37. Zhang Y, Yu G, Xiang Y, et al. Bm-TFF2, a toad trefoil factor, promotes cell migration, survival and wound healing. Biochem Biophys Res Commun. 2010;398(3):559-564.
38. Capon A, Mordon S. Can thermal lasers promote skin wound healing? Am J Clin Dermatol. 2003;4(1):1-12.
39. Zeke T, Morrice N, Vázquez-Martin C, Cohen PT. Human protein phosphatase 5 dissociates from heat-shock proteins and is proteolytically activated in response to arachidonic acid and the microtubule-depolymerizing drug nocodazole. Biochem J. 2005;385(Pt 1):45-56.
40. Wilmink GJ, Opalenik SR, Beckham JT, et al. Molecular imaging assisted optimization of Hsp70 expression during laser-induced thermal preconditioning for wound repair enhancement. J Invest Dermatol. 2009;129(1):205-216.
41. Chang W, Song BW, Lim S, et al. Mesenchymal stem cells pretreated with delivered Hph-1-Hsp70 protein are protected from hypoxia-mediated cell death and rescue heart functions from myocardial injury. Stem Cells. 2009;27(9):2283-2292.

| **Visit Name** | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8** | **V9** |
|---|---|---|---|---|---|---|---|---|---|
| Post-operative day | -7 ±2 | 3 ±1 | 7 ±2 | 14 ±4 | 42 ±6 | 70 ±6 | 98 ±6 | 180 ±14 | 365 ±14 |
| | | | | | | | | | |
| Informed Consent | X | | | | | | | | |
| Physical Exam | X | | | | | | | | |
| Medical History | X | | | | | | | | |
| Assign Screen/ Random No. | X | X | | | | | | | |
| Expert Grader Assessment | | X | X | X | X | X | X | X | X |
| Pain/Sensitivity Assessments | | X | X | X | X | X | X | X | X |
| Photographic Assessment | | X | X | X | X | X | X | X | X |
| AE Assessments | | X | X | X | X | X | X | X | X |
| Dispense Test product | | X | X | | | | | | |
| Collect Test product | | | | | X | | | | |
| Range of Motion | | X | X | | X | | | X | X |

Table 1. Treatment schedule.

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| Informed Consent | Employee or immediate family of either AkPharma or employee or immediate family of investigator or site personnel. |
| 45 – 75 years of age | Pregnant or breast feeding |
| Scheduled for bilateral knee-replacement surgery | Known allergy or hypersensitivity to calcium or phosphorus supplements |
| | Diagnosis of type I or type II diabetes mellitus |

Table 2. Inclusion and Exclusion Criteria

| | | | | |
|---|---|---|---|---|
| Gender | Male | 6 | Female | 14 |
| Race | Caucasian | 18 | Black | 2 |
| Age (years) | Mean | 61.25 | | |
| | Median | 62 | | |
| | Mode | 62 | | |
| | Range | 49 - 72 | | |

Table 3. Patient demographics.

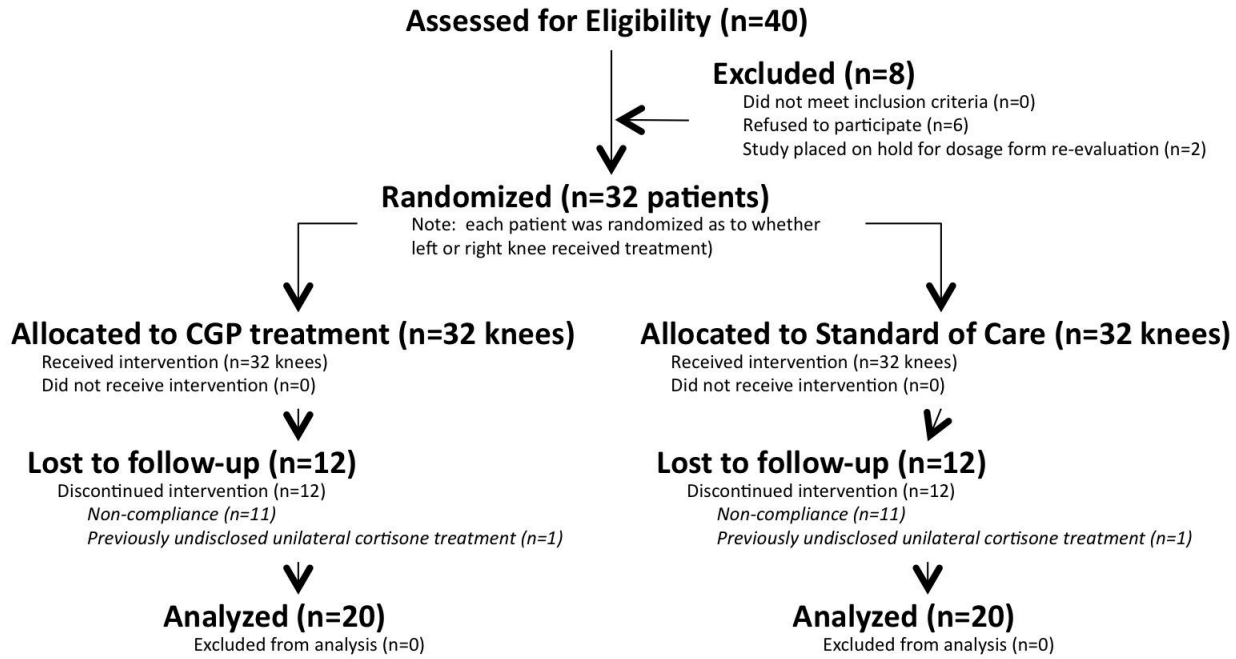


Figure 1. CONSORT flow diagram for patient recruitment and randomization. Patient compliance was assessed by examining the treatment kits for unused product. Those patients found to be non-compliant discontinued the study. Please see text for details.

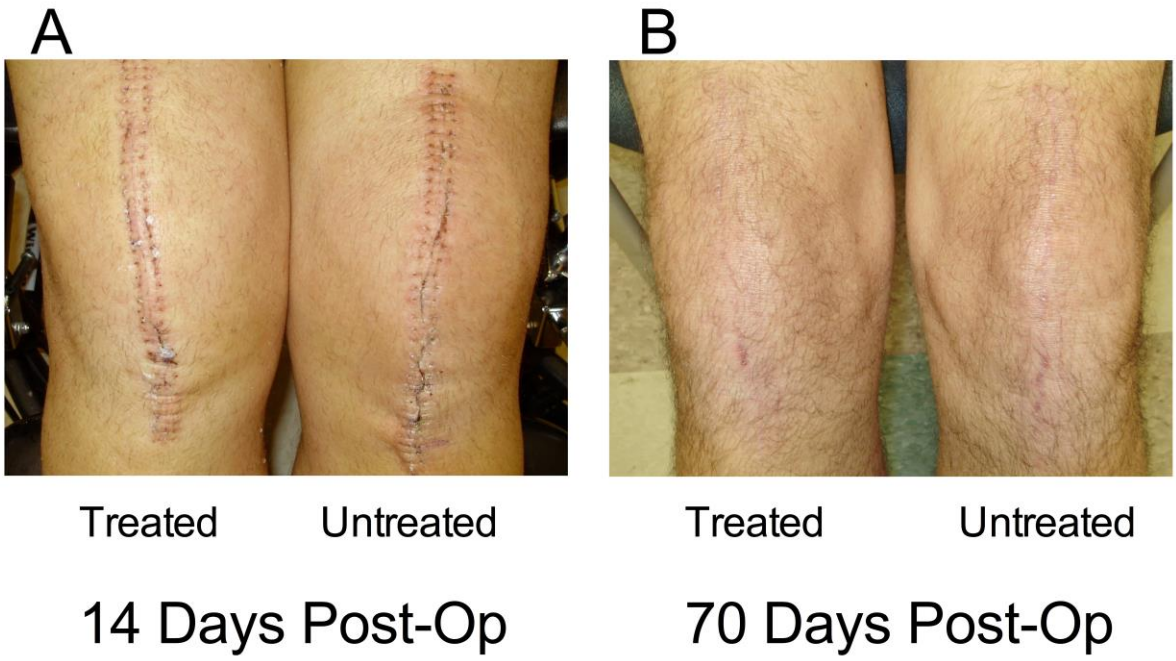


Figure 2. Digital photographs of a single representative patient, at the 14$^{th}$ and 70$^{th}$ post-operative days. At day 14, the appearance of the treated knee is markedly better than the untreated knee. While the difference had diminished somewhat by day 70, there is still a discernable difference between the two.

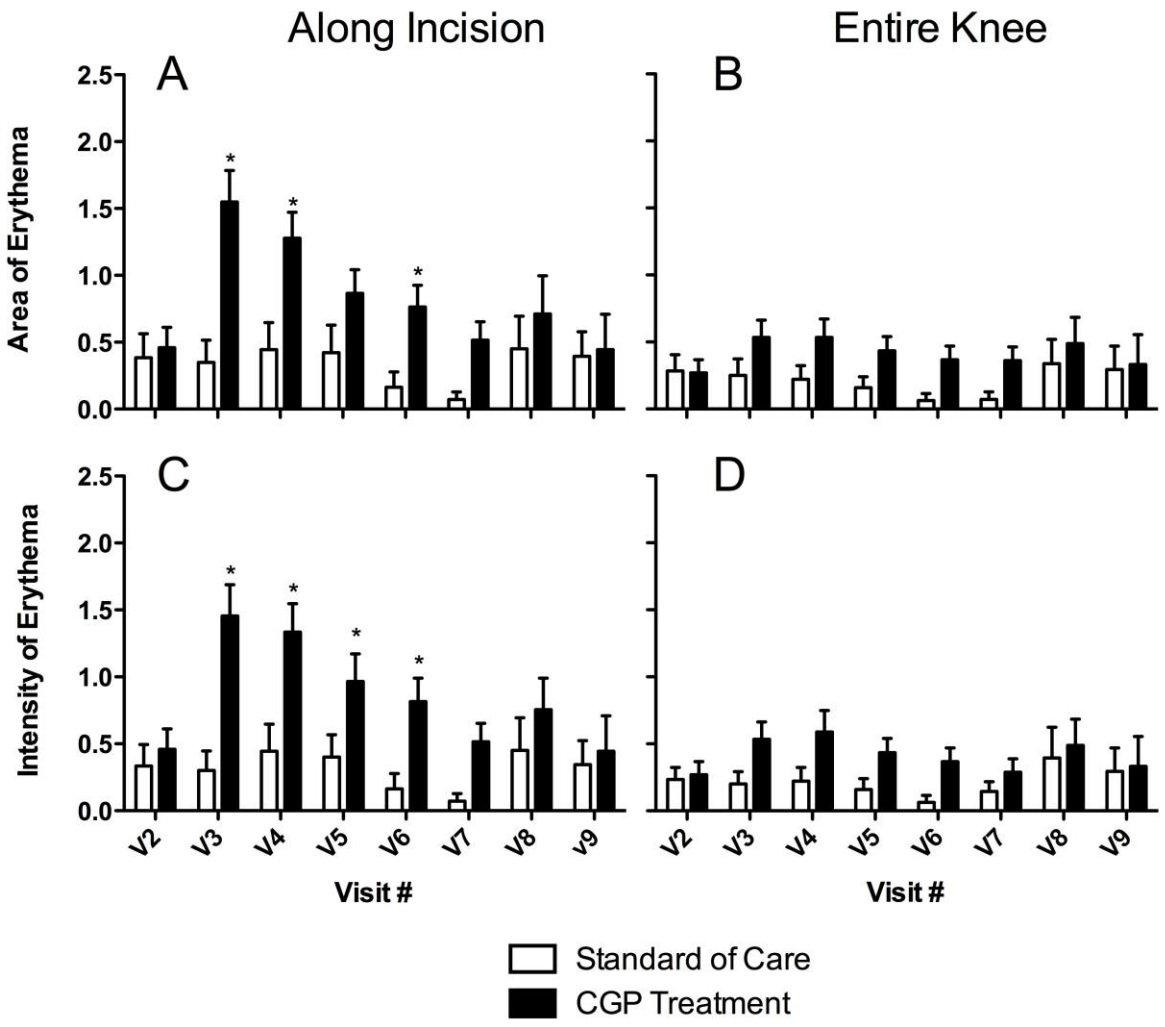


Figure 3. Clinical impression of erythema for all patients. Both area and intensity of erythema along the incision (panels A and C) were markedly and significantly (p=0.003) better (scored higher) in the CGP treated *vs.* the standard of care, particularly during the treatment period (visits 3 through 6). While not so dramatic as the incision results, the area (Panel B; p=0.0019) and intensity (Panel D; p=0.003) of erythema over the entire knee was significantly improved (scored higher) in the treated *vs*. the untreated knee. The "p" values are the overall probability that the results of the study occurred by chance, as calculated by two-way analysis of variance. * indicates $p<0.05$, treated *vs*. untreated, for a single visit.

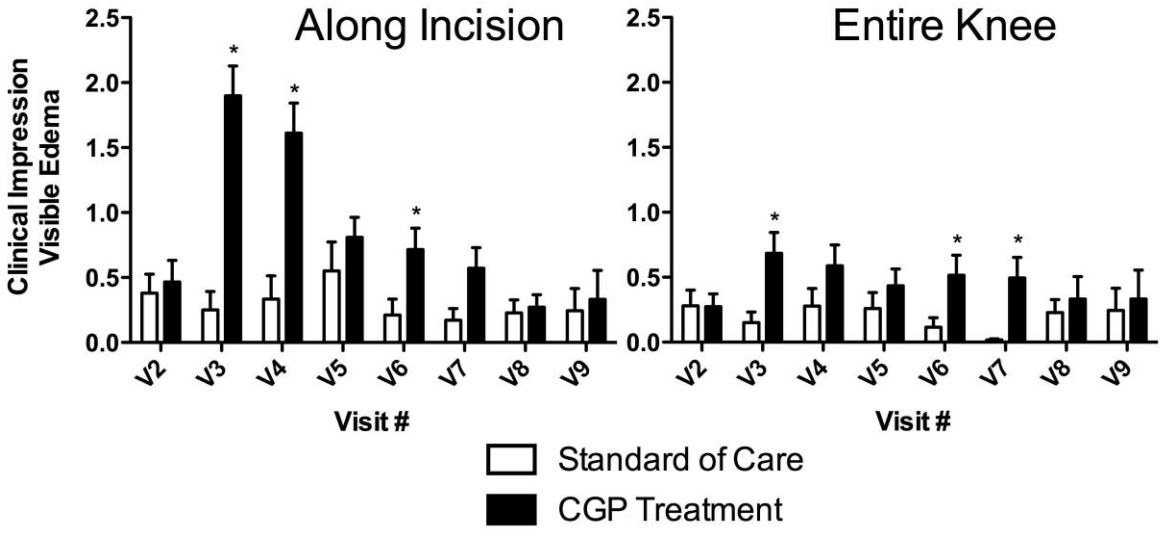


Figure 4. Clinical impression for all patients of visible edema along the incision line (Panel A) and over the entire knee (Panel B). In both measures, the CGP treated knee exhibited significantly less visible edema (scored higher) as compared to the standard of care. Panel A: $p<0.0001$; Panel B: $p=0.0002$. The "p" values are the overall probability that the results of the study occurred by chance, as calculated by two-way analysis of variance. * indicates $p<0.05$, treated *vs*. untreated, for a single visit.

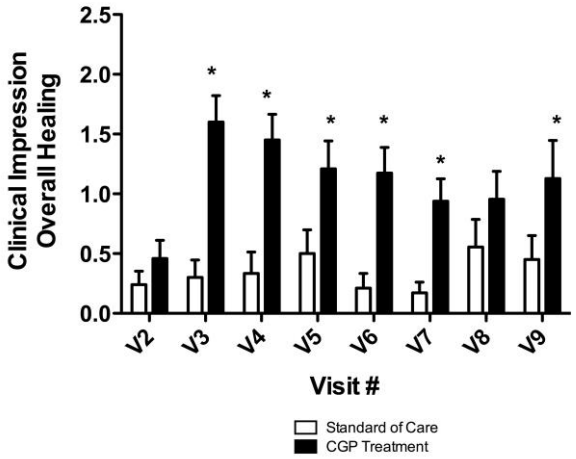


Figure 5. Global clinical impression of wound healing for all patients. Overall, the CGP treated knee healed markedly and significantly faster (scored higher) as compared to the standard of care ($p<0.0001$). The "p" values are the overall probability that the results of the study occurred by chance, as calculated by two-way analysis of variance. * indicates $p<0.05$, treated *vs*. untreated, for a single visit. Open bars - untreated

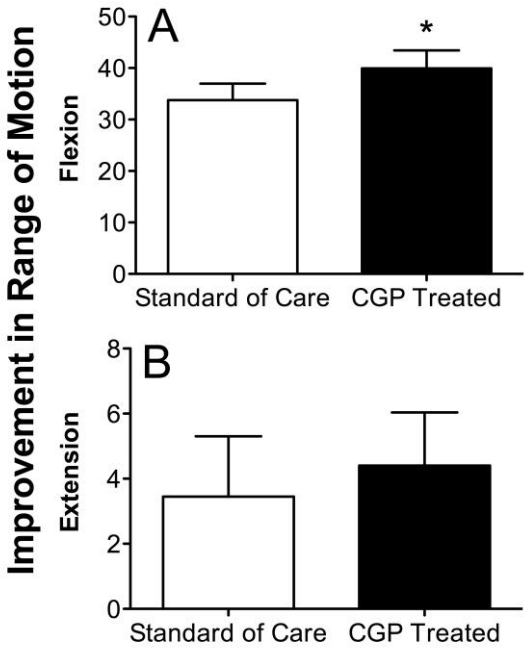


Figure 6. Improvement in flexion (panel A) and extension (panel B) between visit 2 (pre-treatment) and visit 5 (after 6 weeks of treatment). The treated knees gained significantly more flexion during the treatment period (p=0.046). While the treated knees had slightly better extension gains, the difference was not statistically significant (p=0.07). By 6 months, there were no differences between treated *vs.* untreated knees, for either flexion or extension. *=p<0.05 by paired ttest.