Binding and Elution of Proteins Contained in a Naturally Derived Protein Allograft (AlloSpark-GF®) to Commercially Available Bone Grafts

Michael Zimber, PhD[2], Hailey Black[2], Jared Diegmueller[1], Brian Barnes, PhD[1], Bryan Choi[2]

[1] Isto Biologics, Hopkinton, MA 01748
[2] Isto Biologics, Carlsbad, CA 92008

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Abstract

Effective bone regeneration requires coordinated, multifactor signaling delivered with appropriate spatiotemporal control, yet many current therapies still rely on single growth factor approaches with rapid release profiles that do not recapitulate native healing biology. This study characterizes AlloSpark-GF®, a novel allograft-derived, multigrowth factor formulation, with respect to its protein composition and its interaction with a range of clinically relevant bone graft scaffolds and compares its delivery behavior to INFUSE (rhBMP-2). Proteomic analyses demonstrate that AlloSpark-GF® contains a diverse set of osteogenic, angiogenic, and chemotactic proteins consistent with pathways involved in bone repair. Across multiple scaffold types, AlloSpark-GF® exhibits robust binding and a generally controlled, sustained release profile, with scaffold-dependent variation reflecting differences in material composition and affinity. In contrast, rhBMP-2 delivered from a collagen-based system displays a more rapid, bolus-dominated release. Collectively, these findings indicate that AlloSpark-GF® provides a signaling environment with more prolonged local presentation, which may better approximate the physiological processes underlying bone healing compared to single-factor approaches.

Introduction

Reliable bone healing remains a significant challenge in orthopedic surgery, particularly in cases requiring fracture repair, spinal fusion, or revision procedures. Although many fractures and fusion procedures heal successfully without additional intervention, approximately 5–10% progress to delayed union or nonunion and require further surgical treatment.[1-3] These revision procedures are associated with increased morbidity, prolonged recovery, and substantial healthcare expenditures, placing a significant burden on both patients and healthcare systems.

Autologous bone grafting, particularly iliac crest bone grafting (ICBG), continues to be a highly effective standard for effective bone repair and regeneration, but the incorporation of autograft is not always without challenges.[4] Harvesting ICBG requires a secondary site with the risk of morbidity and pain, increased operating room times, and possibility of lack of sufficient graft material.

Advancements in bone tissue engineering and regenerative medicine have expanded the range of materials and technologies available to support bone regeneration. Current approaches include synthetic biomaterials (e.g., polymers, ceramics, and metals), allografts and demineralized bone matrices, osteoinductive biologics and growth factors, and cell-based therapies, each offering distinct advantages and limitations with respect to osteoconductivity, osteoinductivity, biocompatibility, handling characteristics, and clinical performance.[5,6]

One therapy that has gained popularity is recombinant human bone morphogenetic protein 2 (rhBMP-2), commercially marketed as INFUSE Bone Graft (Medtronic, Memphis, TN).[7] BMP-2 is a member of the transforming growth factor-beta (TGF-β) superfamily of proteins and, along with the other bone morphogenetic proteins, has been shown to play an important role in the development of bone and cartilage.[8]

INFUSE recombinant rhBMP-2 supplied as a lyophilized powder that, following reconstitution, is adsorbed onto an absorbable collagen sponge (ACS) composed of type I bovine collagen prior to implantation. In the United States, INFUSE is FDA-approved for specific spinal fusion procedures,

including certain ALIF, OLIF, and TLIF procedures, as well as for the treatment of selected acute open tibial shaft fractures stabilized with intramedullary nail fixation and certain oral-maxillofacial and dental bone regeneration procedures.[9]

While BMP-2 is an important factor in bone healing, the regenerative process is complex and involves numerous growth factors with critical and coordinated biological activities including vascular endothelial growth factor (VEGF), insulinlike growth factor 1 (IGF-1), bone morphogenetic protein 7 (BMP-7), the fibroblast growth factor (FGF) family, platelet derived growth factor (PDGF), and other members of the transforming growth factor (TGF) family.[10]

AlloSpark-GF® (Isto Biologics, Hopkinton, MA) is a novel bone allograft-derived supplemental comprised of naturally occurring proteins. It is supplied as a lyophilized powder to be reconstituted and used with a physician's scaffold of choice for bone forming applications. In this study, we characterize the protein composition of AlloSpark-GF® for the presence of potentially beneficial factors in the context of bone regeneration. Also, we evaluate the binding and elution characteristics of the protein present in AlloSpark-GF® on several commercially available bone graft materials including synthetic bone graft substitutes (BGS), collagen and demineralized bone allografts.

Methods & Materials

Proteomics via Liquid Chromatography –Mass Spectrometry (LC-MS/MS)

Tandem mass spectrometry (LC–MS/MS) was used to identify and quantify the global protein profile of AlloSpark-GF®. Samples were reconstituted, washed, enzymatically digested, and resulting peptides were analyzed by mass spectrometry and matched against an established proteomic library. Targeted LC–SRM mass spectrometry was then used to quantify specific growth factors. For each peptide, the total integrated peak area from the top three transitions was averaged, and when multiple peptides represented a single target, their average areas were combined. These values were normalized to peptides from yeast alcohol dehydrogenase (internal standard, 25 fmol) spanning a range of ionization efficiencies to obtain relative concentrations. Absolute quantities were derived from total protein measurements.

Scaffold Binding and Elution

Bone scaffold types representing demineralized bone allograft, collagen-based, mineral, and synthetic categories of commercially available bone grafts were evaluated for AlloSpark-GF® binding and elution characteristics. AlloSpark-GF® prepared according to the Instruction for Use (IFU) was added to the scaffolds such that each scaffold completely absorbed the liquid volume. The scaffold was treated with AlloSpark-GF® for 15 minutes at room temperature and the scaffold was washed with 30 mL of 0.9% saline solution at room temperature. The saline wash was collected and prepared for subsequent protein measurement to determine the initial amount of AlloSpark-GF® bound to the scaffold.

Scaffolds were taken directly from the binding portion of the study, placed into 30 mL of physiological saline and incubated at 37°C. At predetermined timepoints of 2, 4, 6, 24, 48 and 72 hours following binding, the saline solution was completely removed and replaced with an equivalent volume of fresh saline. Collected solution samples were dialyzed against distilled

water at 4°C using 2 kDA MWCO dialysis cassettes (ThermoFisher Scientific, Waltham, MA), frozen and lyophilized. Samples were reconstituted in distilled water, and total protein was quantified using the BCA Protein Assay Kit (ThermoFisher Scientific, Waltham, MA). Protein measurements were used to calculate the percentage of protein initially bound to the scaffolds and the percentage of bound protein eluted over time.

Results

Proteomics via Liquid Chromatography–Mass Spectrometry

Global LC–MS/MS analysis identified 623 human proteins at 99% confidence for peptide identifications and 95% confidence for protein identifications. The analysis was able to detect various osteogenic, angiogenic, mitogenic, chemotactic and morphogenic proteins. LC-SRM was used to further measure specific proteins, and their concentrations can be found in Table 1.

Target	Full Name	[Target] (ng/g ProteiOS)	SD
aFGF	acidic fibroblast growth factor	68,695	10,571
bFGF	basic fibroblast growth factor	454,922	157,278
BMP-2	bone morphogenetic protein 2	41,786	25,379
BMP-4	bone morphogenetic protein 4	12,040	1,396
BMP-6	bone morphogenetic protein 6	11,149	4,924
BMP-7	bone morphogenetic protein 7	35,459	7,191
BMP-9	bone morphogenetic protein 9	72,950	19,561
IGF-1	insulin-like growth factor 1	31,993	9,600
OPG	osteoprotegerin	51,896	15,077
OPN	osteopontin	18,918	1,033
PDGF-BB	platelet derived growth factor BB	97,456	30,045
TGF- 1	transforming growth factor 1	37,548	9,901
VEGF	vascular endothelial growth factor	33,752	1,969

Table 1 – Proteins identified in AlloSpark-GF® via LC-SRM

AlloSpark-GF® Binding and Elution

AlloSpark-GF® successfully bound to all types of graft scaffolds representing demineralized bone allograft, mineral, synthetic and collagen-based categories with a mean initial binding efficiency of 73.8% (SEM=4.7) following a 15-minute incubation period (Figure 1A). At 2 hours post-binding, over 91.5% (SEM=1.6) of the initially bound AlloSpark-GF® was retained by the scaffolds. The elution profile of AlloSpark-GF® from most of the scaffolds presented a biphasic nature consisting of an initial period of rapid, burst-type release between 0-6 hours post-binding, but still retained protein with a mean of 75.5% (SEM=5.4) of the initially bound protein. Elution of protein from these scaffolds continued over the duration of the experiment, and was characterized by a slower, more sustained release, with a mean of 50.7% (SEM=9.3) of initially bound AlloSpark-GF® retained at 72 hours post-binding (Figure 1B).

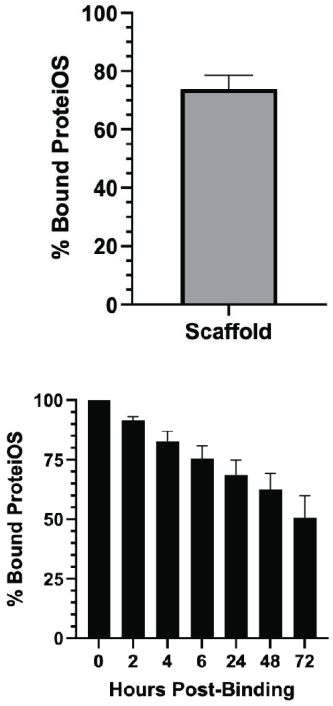

Figure 1. A. Range of AlloSpark-GF® binding to various bone graft scaffolds. AlloSpark-GF® initially bound to all scaffolds (n= 11) with a mean efficiency of approximately 74% of applied AlloSpark-GF®. B. Protein elution profile of AlloSpark-GF® protein from bone graft materials. Eight scaffold types were evaluated over a 72-hour period at 37C. Data expressed as mean +/- SEM.

Discussion

As the fields of science and medicine evolve, numerous products and therapies have emerged to supplement or provide alternatives to autogenous bone grafting. Among these, recombinant human BMP-2 (INFUSE) represented a major advance and has demonstrated reliable fusion efficacy comparable to autograft in many clinical settings.[11]

Clinical experience has highlighted the importance of dose control and anatomical context, as supraphysiologic BMP-2 signaling may contribute to complications including local inflammation, ectopic bone formation, radiculitis, soft-tissue swelling, and osteolysis.[12-15] Large retrospective cohort studies suggest that higher rhBMP-2 doses are associated with increased complication rates, whereas lower-dose protocols can reduce adverse events without clear loss of fusion efficacy.[16-18]
These findings have prompted a shift away from high-dose, single-factor osteoinductive approaches toward lower-dose regimens, combinatorial growth factor strategies, and multifactorial signaling paradigms that better recapitulate endogenous bone healing while minimizing adverse events.[12,16,19]

Bone tissue has a high intrinsic regenerative capacity and can heal without scar formation under appropriate biological conditions, a property established in foundational bone biology and tissue engineering literature.[3,4] Bone repair proceeds through coordinated phases consisting of inflammation, angiogenesis and soft callus formation, osteogenesis and hard callus formation, and remodeling, each regulated by distinct but overlapping signaling pathways and is schematically outlined in Figure 4.[5,20] These phases are controlled by multiple growth factors that act in a defined spatiotemporal sequence, including VEGF, PDGF, FGF, BMP family members, TGF-β, and IGF-1, which collectively regulate cell recruitment, vascularization, differentiation, and matrix remodeling.[10,21]

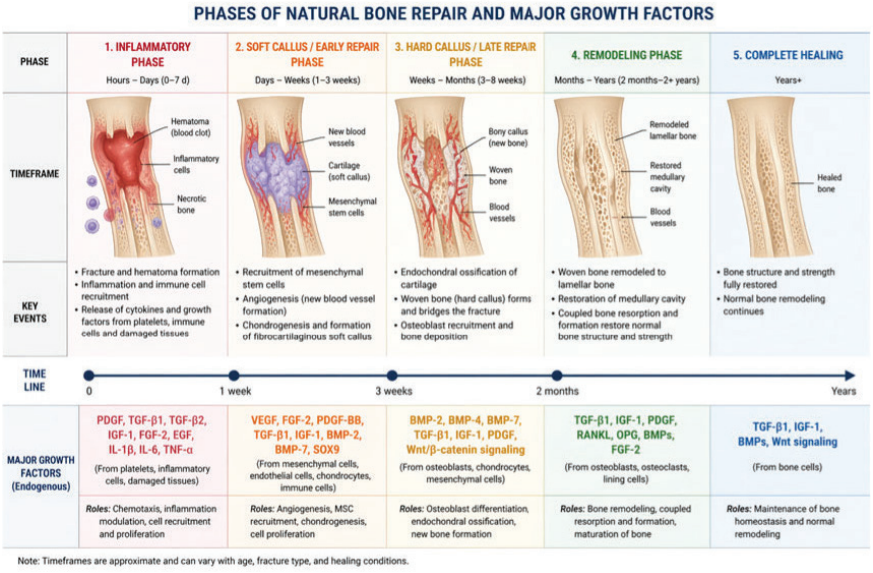

Figure 4. Spatiotemporal phases of bone healing and associated growth factors. Image generated using DALL·E (OpenAI), 2026. No external or copyrighted images were used or modified. Content reviewed and curated by the authors.

Experimental studies have demonstrated that delivery of multiple growth factors, particularly when angiogenic and osteogenic signals are combined, promotes bone regeneration more effectively than single-factor approaches.[21,22] In a rat fracture model, combined IGF-1 and TGF-β1 delivery enhanced healing relative to controls, while co-delivery of VEGF and BMP-2 increased mesenchymal stem cell recruitment and bone formation compared with single-factor treatment.[23,24] More recently, incorporation of angiogenic and tissue maturation signals has been shown to further enhance regeneration, as controlled delivery of VEGF and PDGF in combination with BMP-2 improved vascularized bone formation and mechanical properties compared with BMP-2 alone in a composite injury model.[25,26]

There is also substantial evidence that augmentation of BMP-2 with complementary biologic signals enables effective bone regeneration at lower BMP-2 doses. Angiogenic factors such as VEGF and PDGF enhance BMP-2-mediated bone formation by improving vascularization, progenitor cell recruitment, and tissue maturation, thereby reducing the amount of BMP-2 required to achieve comparable regenerative outcomes.[24,25,27] Similarly, Egashira et al. demonstrated that supplementation of low-dose BMP-2 with bone marrow concentrate or plateletrich plasma, both of which provide heterogenous signaling factors, significantly increased bone formation compared with low-dose BMP-2 alone in a calvarial defect model. [28] These findings suggest that BMP-2 efficacy can be enhanced through complementary biological signals, supporting dose-sparing strategies that maintain or improve regenerative outcomes while reducing reliance on single factor high-dose BMP-2.

In addition to growth factor selection, release kinetics are a critical determinant of therapeutic efficacy. Sustained and controlled delivery has been shown to be superior to single high-dose administration, particularly for BMP-2, where rapid release from conventional carriers can generate supraphysiologic local concentrations and associated complications.[19,22] Delivery systems that provide sequential angiogenic and osteogenic signaling have been shown to improve regenerative outcomes by more closely replicating the natural healing process, a concept further supported by dual-delivery and temporally controlled release strategies.[21,29]

The native bone extracellular matrix provides a model for growth factor regulation, serving as a reservoir through interactions with glycosaminoglycans and structural proteins that regulate growth factor sequestration, presentation, and bioactivity.[30,31] During bone remodeling, matrix-bound factors such as TGF-β and IGF-1 are released through osteoclast-mediated resorption and proteolytic matrix degradation, coupling bone resorption to subsequent bone formation.[20,32] Because many growth factors have relatively short physiological half-lives following release, local retention and controlled presentation are important determinants of their bioavailability and biological activity. Understanding these mechanisms may help improve the retention and biological activity of multiple growth factors delivered in bone grafts and regenerative therapies.

In this study, we examined the protein composition and release kinetics of AlloSpark-GF® in an *ex vivo* model of protein elution. The results highlight two key parameters that support the use of AlloSpark-GF®, namely the presence of multiple growth factors and the slow, sustained release of proteins. Via mass spectrometry, multiple growth factors were identified in AlloSpark-GF® that are associated with

bone growth, angiogenesis, wound healing, and tissue regeneration. AlloSpark-GF® release from bone graft scaffolds is generally controlled and sustained. Overall, most scaffolds retain AlloSpark-GF® with minimal early elution, reflecting the influence of scaffold composition on binding affinity and release kinetics and supporting sustained presentation of multiple signaling molecules across different materials.

Previously, Govil et al. described the binding of several growth factors present in AlloSpark-GF® to a synthetic bone graft scaffold composed of β-TCP, bioglass, and type I collagen following application of AlloSpark-GF®.[33] After a 15-minute incubation at room temperature, total protein binding exceeded 98% of the applied protein. Binding of aFGF, bFGF, VEGF, PDGF-BB, TGF-β1, BMP-2, BMP-4, BMP-7, and OPN exceeded 80%, with most growth factors above 90%, including BMP-2. In the same model, rhBMP-2 exhibited a bolus release from its ACS carrier scaffold, with approximately 50% of the applied protein released into the surrounding solution within 8 hours and 72% released over 3 days in solution. AlloSpark-GF® exhibited a more controlled release profile from the synthetic bone graft substitute, with only 14% of the total protein released by 72 hours and no significant initial bolus release observed.

While the results of this study highlight both the multifactor composition of AlloSpark-GF® and the potential benefits of delivering multiple growth factors in a clinical setting, they also emphasize the importance of scaffold selection, as delivery platforms differ in their ability to retain and release bioactive proteins. Importantly, these findings should not be interpreted as favoring a single scaffold type, as both biologic and synthetic graft materials offer distinct advantages and limitations that should be considered in the context of the intended clinical application.

Although additional translational studies are required to fully understand clinical efficacy, given the current knowledge of bone healing and regenerative medicine, multiple growth factor-containing AlloSpark-GF® may offer a reliable and predictable bone-forming strategy without the adverse event potential associated with technologies that use supraphysiological levels of a single osteoinductive protein.

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