Mineral Ion Release and Antimicrobial Properties of Biointegrative Implants

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INTRODUCTION: Mechanical strength and durability are key factors for achieving successful bone osteosynthesis when using bone fixation implants[1]. Traditionally, most implants were made of metals, however, metal fixation hardware introduces inherent risks such as implant migration, bone tissue loss, irritation, infection and acute or chronic inflammation, all of which pose the frequent need for hardware removal[2]. The use of biomaterials for orthopedic fixation can provide a solution to these limitations[3]. The biointegrative, mineral fiber-reinforced implants used in this study offer strong bone fixation while avoiding permanent hardware and enabling complete restoration of native anatomy. The implants are composed of continuous reinforcing mineral fibers, comprised of elements found in native bone (SiO_2, Na_2O, CaO, MgO, B_2O_3, and P_2O_5; approximately 50% w/w), and bound together by polymer resin [poly (L-lactide-co- D,L-lactide), PLDLA] (Figure 1A-C)[4,5]. The mineral fiber component is similar in composition to bone fillers such as bioactive glasses that are known for their osteoconductive properties. Bioglass is the most widely known bioactive material, made typically from different ratios of Na_2O-CaO-MgO-P_2O_5-SiO_2 elements. Bioglass is known for its unique ability to form a mechanically strong bond with bone[6,7]. Unlike other biocomposites and bioresorbable orthopedic implants which rely solely on the polymer content for mechanical performance, the biointegrative implants leverage both the biological, as well as the mechanical advantage of the mineral fiber matrix. Their bone ingrowth and regeneration have been demonstrated in preclinical *in vivo* models and thought to be related to the high mineral content of these implants. Furthermore, there are well established studies showing the antimicrobial properties of bioactive glasses. Bone injuries, particularly open fractures, can cause microorganisms to enter the body and cause osteomyelitis (bone infection), with *Staphylococcus aureus* (*S. aureus*) being the most prevalent pathogen causing osteomyelitis in adults[8]. The purpose of this study was to evaluate the early ion release of the major 4 elements of the mineral component in biointegrative mineral fiber-reinforced implants. This study also aimed to examine the hypothesis that the high mineral content of the biointegrative implants can benefit the local tissue environment with bacterial inhibition properties.

METHODS: Ion Release Study: Biointegrative Cannulated Trimmable Fixation Nails (CTFN) 3mm x 50mm (OSSIOfiber®, Ossio Ltd.) (Figure 1D) were incubated in 30 mL of 0.9% saline at physiological conditions (37°C) with slightly acidic pH of 5.5. Nails ranged in weight from 0.41g – 0.42g. One nail was incubated in each solution sample, with 3 replicate samples at each timepoint of 3hrs, 12hrs, 1, 4, 7, 11, 15, 21 and 30 days. Saline samples served as negative control (blank). pH levels were measured at each time point. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analysis was performed on the degradation media to evaluate the amount of mineral ions: (Silicone (Si), Calcium (Ca), Magnesium (Mg) and Phosphate (P)) released into the media at each time point throughout the 30-day period.

Bacterial Inhibition Study: Three test groups were prepared, for similar surface areas: **1.** Four Biointegrative OSSIOfiber® CTFN 3mm x 50mm pins, 10mm each, **2.** Nine 1.8mm titanium K-Wires (Kirschner Wires) 10mm each, and **3.** Two PLDLA pins trimmed from CTFN 4mm x 70mm, 15 mm each. All samples were incubated with 0.1mL of *S. aures* suspension in Fluid A (USP), to obtain about 10^4 CFU (Colony Forming Unit) per tube, in 37°C for 1,2,5 and 7 days. A fourth group of Fluid A samples served as positive control. At each time point, serial 10-fold dilutions were performed and filtered through a 0.45µm filter membranes. The filters were placed on Tryptic Soy Agar (TSA) solid plates, which were incubated at 30-35°C for up to 5 days. Following incubation, the total number of colonies on each plate were counted and recorded. Results are expressed as CFU per sample.

RESULTS: Ion Release Study: An increase in the test group media pH level observed throughout the 30-day period from 5.5 to 8.78. In fact, pH level peaked after 15 days (8.87) and stayed almost steady for the next 15 days, thus keeping the alkaline surroundings (Figure 2A). ICP-OES analysis indicates an increase in all the 4 tested ion concentrations in the degradation media. The initiation time point and detected rate of ions release was different between the ions. While Si started its release in the media as soon as 3 hrs. following incubation, P ions were evident only on day 4. The media concentration of Si remained the highest at the end of the study (day 30, 70.33 ±6.98 mg/L) (Figure 2B), while P concentration reached the value of 2 ±0 mg/L, already after 15 days, and stayed steady (Figure 2E). Ca and Mg ion release demonstrated an intermediate trend of initiation point and rate reaching their highest concentrations at the 30-day (21 ±0.71mg/L and 8.67 ±0.41 mg/L respectively) (Figure 2C&D).

Bacterial Inhibition Study: The initial count of *S. aures* was about 10^4 CFU for all groups. An increase in bacteria count was measured during the first 2 days in all groups, explained by the growth support conditions carried out on the substrate of the TSA plates. From this point until day 5, the numbers continued to increase, however at a different rate for each treatment. The control and titanium implant groups showed an intermediate rate of *S. aures* CFU growth. The PLDLA implant group showed the highest rate of *S. aures* CFU growth. The biointegrative implant group demonstrated an overall lowest level when compared to all treatments, with significantly decreased CFU count at day 5 as compared with the PLDLA group. From day 5 to day 7, due to natural mortality of the bacterial populations, the PLDLA group showed a slight decrease of the bacteria colonies number, while the titanium and the biointegrative groups reached saturation. At day 7, the bacterial measured CFU in the biointegrative group was significantly lower compared to all other treatment groups (Figure 3).

DISCUSSION: These pH and mineral ion release results suggest that the mineral content in the biointegrative OSSIOfiber® implants mitigates the pH drop typically associated with polymer degradation. This is consistent with the hypothesis that the high mineral content of these implants can benefit the local bone healing environment. The sustained alkaline environment, in turn, appears to inhibit the maturation of *S. aureus* biofilms and reduces the likelihood of bacterial colonization and infection.

SIGNIFICANCE: The results of this study demonstrate that biointegrative fiber-reinforced implants provide their local environment with a steady supply of key mineral ions in the early days following simulated implantation in the body. These mineral components have been associated with optimal bone healing and regeneration. By inhibiting bacterial growth and decreasing the risk of infection, such implants may further prevent the need for hardware removal procedures, which by itself carries a risk of complications, such as secondary bacterial contamination.

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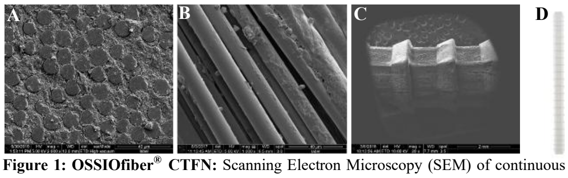

Figure 1: OSSIOfiber® CTFN: Scanning Electron Microscopy (SEM) of continuous mineral fibers surrounded by polymeric material, in cross section **(A),** longitudinal section **(B)** and implant ribs **(C)**. CTFN implant image (**D**).

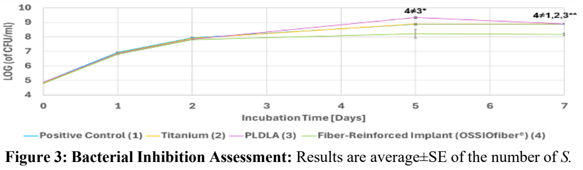

Figure 3: Bacterial Inhibition Assessment: Results are average±SE of the number of *S. aures* colonies (expressed as LOG of CFU/ml) at different time points thorough 7 days.

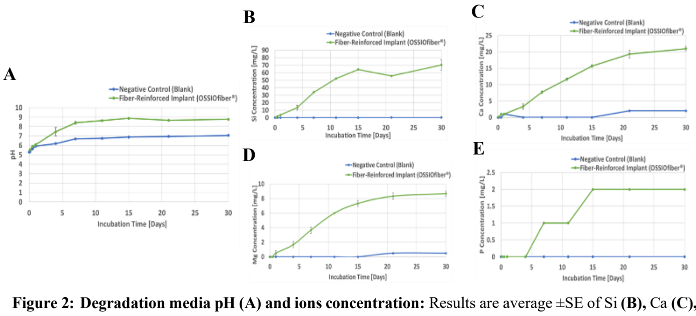

Figure 2: Degradation media pH (A) and ions concentration: Results are average ±SE of Si **(B),** Ca **(C),** Mg **(D)** and P **(E)** concentration thorough 30 days.