

# A Pre-Clinical Evaluation of a Novel Calcium Phosphate Bone Cement: DirectInject®

## BACKGROUND

The primary objective of cranioplasty techniques is to restore the form and function of the calvaria. Given the compatibility and general availability, autologous bone has historically been considered the “gold standard” in restoring cranial defects. However, due to the rate of resorption, challenges with contouring, and donor site morbidity, alloplastic materials have become a significant surgical tool.<sup>1</sup>

Calcium phosphate based hydroxyapatite “bone cements” have become a more wide-spread material in craniofacial applications. As a synthetic analog to the naturally occurring mineral component of bone, such products serve as an ideal bone void filler due to the ease of application and ability to conform, comparable strength and architectural structure to bone, and overall biocompatibility and ability to osteointegrate.<sup>2-4</sup>

DirectInject® consists of a sterile dual paste system which is calcium phosphate based. Upon injection from a double barrel delivery syringe system through the mixer-cannula, the two pastes form a moldable cement. The injected cement paste will harden under normal body conditions in a wet field to form hydroxyapatite.

The purpose of this study was to determine an overall biocompatibility safety profile for DirectInject® when compared to its marketed predicate device (HydroSet®, Stryker Craniomaxillofacial, Kalamazoo, MI) in multiple rabbit osteotomy models.



Image 1: DirectInject® double barrel delivery syringe system

## METHODS

For assessment of local tissue effects and biocompatibility, the standard guidance documents were utilized per Guidance for Industry and FDA. Class II Special Controls Guidance Document: Resorbable Calcium Salt Bone Void Filler Device, dated June 2, 2003, was utilized for the animal performance testing conducted to support the Indications for Use and performance characteristics of Stryker DirectInject®. All animal testing was performed with finished, sterilized product

in compliance with Good Laboratory Practices (GLP) and ISO 10993-1 Biological Evaluation of Medical Devices. Table 19-1 provides a summary of the test design.

TABLE 19-1 – ANIMAL PERFORMANCE TESTING

Animal Testing	12-week cranial	12-week femoral	52-week femoral
Model	Rabbit		
Age of animal	Skeletally mature		
Defect Size	Critical		
Samples	<ul style="list-style-type: none"> <li>• Test Article: DirectInject®</li> <li>• Control Article: HydroSet®</li> </ul>		
Time Points	4 & 12 week	4 & 12 week	8, 26 & 52 week
Analysis	Local tissue response, technical and biological performances evaluated post-operatively by macroscopic, radiographic, histologic and histomorphometric analyses of the implant sites and surrounding tissue.		

## Preclinical Observations

The rabbits were monitored until complete recovery from anesthesia then returned to their cages. The animals were observed once daily for any clinical abnormality. In case of detection of clinical abnormality, a complete veterinary clinical examination was performed and observations recorded. Animals showing severe signs of debility or morbidity were examined by a veterinarian and euthanized if needed. In cases of death or euthanasia before termination of the study due to surgical procedures, an autopsy was performed and implantation sites were sampled, identified, and fixed in 10% buffered formalin.

## Histopathological Observations

Semi-quantitative evaluation of the local tolerance was performed according to ISO 10993-6 standard involving evaluation of fibrin, resorption or osteolysis, necrosis, tissue degeneration, signs of infection, inflammatory reaction (heterophils, lymphocytes, plasma cells, macrophages, giant cells), fibrosis and any other relevant parameters. The IRS (Irritant Ranking Score) was calculated. The lymph nodes were evaluated for the presence of inflammatory reaction and the presence of degradation articles or migration of particles derived from articles. The performance was qualitatively and semi-quantitatively assessed by analyzing anchoring characteristics, osteointegration, bone neoformation and osteoconduction, osteoblastic and osteoclastic/giant cells, bone density, cartilaginous tissues, fibrous encapsulation and remodeling processes, implant migration, material degradation, neovessels, bone marrow and any other relevant observations.

## Histomorphometrical Analysis

Histomorphometric analysis was conducted by digitalizing and examining slides with a ZEISS AXIOSCOPE microscope (magnifications of x5, x10, x25, and x40) equipped with a color images analyzing system (software SAMBA IPS 4.24, SAMBA TECHNOLOGIES, France). The percentage of bone in-growth (along defect margin), bone to implant contact and biodegradation was calculated and statistically compared

between groups. The rate of degradation was analyzed at the final time points per study.

## RESULTS

### Twelve Week – Rabbit Cranial Model

A 12-week cranial rabbit study was performed comparing DirectInject® to HydroSet® (control) to evaluate the local effects of the product. The purpose of this GLP study was to evaluate the local tissue effects and the performance (including bone healing response and material degradation characteristics) of DirectInject® following cranial implantation in a rabbit skull defect model.

Twenty-nine rabbits were implanted in bilateral cranial sites (critical sized defects of approximately 8 x 15mm) with the control or the test article for 4 and 12 weeks. The number of sites was selected to comply with the ISO 10993-2 standard.

The local tissue effects were evaluated using macroscopic and histological analyses. The performance was quantified using histomorphometrical analyses and the values obtained at each time-period were statistically compared.

Macroscopically, no abnormal local tissue effect was observed at termination 4 weeks and 12 weeks after implantation. Histologically, the irritant ranking score showed that the test article was not locally irritated when compared with the control article at 4 and 12 weeks. No signs of implant-induced osteolysis were detected in the two groups (locally at the level of the osseous implantation site and the underlying brain). No test or control article-related toxic effects were observed.

In terms of performance, the test and the control articles yielded comparable bone healing response. No material degradation was observed over time for the test or control article.

In conclusion, under the experimental conditions of the study, the test and control articles did not induce any local adverse tissue effects. Bone healing characteristics were equivalent and satisfactory around the two articles which were not degraded after 12 weeks (Figure 1).

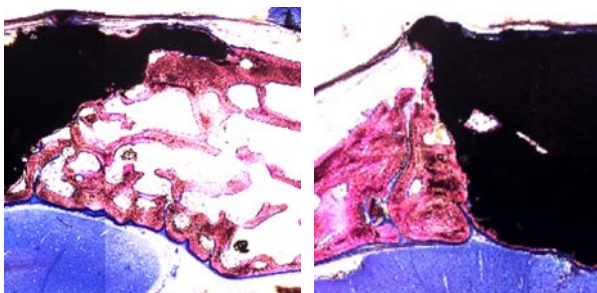


Figure 1: Representative photomicrographs showing local tissue response and marginal defect bone healing (osteointegration) of the control (left) and test (right) articles at 12 weeks in a cranial model (2X magnification).

### Twelve Week - Rabbit Femoral Model

A 12-week femoral rabbit study was performed comparing Stryker DirectInject® to HydroSet® (control) to evaluate the local tolerance including the bone healing response following implantation in the medial femoral condyle of the rabbit (cylindrical defects of 5mm diameter, 10mm length). The

rabbit model is well characterized and has historically been used in femoral implant studies. The number of animals was determined to comply with ISO 10993-6 standard (April, 2007).

Twenty four rabbits including four reserve rabbits were implanted at one site in each femoral condyle with the test article or the control article for 4 and 12 weeks (ten sites per article and per time period). The local effects of the test article after 4 and 12 weeks of implantation were compared with the control article using macroscopical and histological analysis. Moreover the material degradation and bone healing response was quantified using histomorphometrical analysis and the test and control groups were compared statistically.

Macroscopically, no biological difference was observed between both articles after 4 and 12 weeks of implantation. Microscopically after 4 and 12 weeks of implantation, the irritant ranking score (IRS) showed that the test article was not locally irritant as compared to the control article. No signs of undesirable local reaction were observed in the two groups. The two articles were well osteointegrated (Figure 2) at the margins of the defect. In terms of performance, the test and the control articles did not show a statistical difference in terms of bone healing response. The rate of degradation between 4 and 12 weeks for the control and test articles was situated within the same range with respectively 8.7% and 7.8%. A statistically significant progression of degradation process ( $p < 0.05$ ) between 4 and 12 weeks was established for the test and control articles.

Under the conditions of this study, local tolerance including bone healing in response to the test article was considered equivalent to the control article after 4 and 12 weeks. The rate of degradation between 4 and 12 weeks was considered as biologically equivalent for the control and test articles.

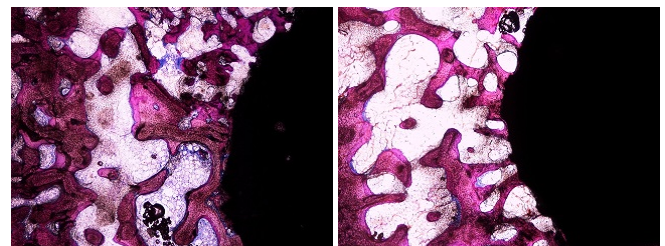


Figure 2: Representative photomicrograph showing local tissue response and marginal defect bone healing (osteointegration) of the control (left) and test (right) articles at 12 weeks in a femoral model (2X magnification).

### Fifty Two Week - Rabbit Femoral Model

A 52-week femoral rabbit study was performed comparing Stryker DirectInject® to HydroSet® (control) to investigate the biological residence and histological effects (including the local tissue effects and the bone healing performance) following implantation in the femoral medial condyle of the rabbit. The number of animals was determined by the Study Sponsor to comply with ISO 10993-6 standard when the two different defects, cylindrical and conical, are analyzed together. The conical defects are particularly well adapted to the analysis of the performance and to the evaluation of the articles resorption as different diameters of defect can be

analyzed using transversal sections performed at different depths.

Fifty-six rabbits (including eight reserve animals) were implanted at one site on each femoral condyle with the test article or the control article for three time-periods: 8, 26 and 52 weeks. Cylindrical (5mm diameter, 10mm length) or conical (4mm diameter, 10mm length) femoral defects were created (eight sites per article, per type of defect and per time-period). The biological residence (article migration and material degradation), the local tissue effects and the bone healing performance (through parameters of osteoconduction, osteointegration, bone density or bone remodeling) of the test article at 8, 26 and 52 weeks of implantation was compared to the control article in the two types of defects (cylindrical and conical defects) using macroscopical, histological and histomorphometrical analysis.

Macroscopically, no biological difference and no abnormal local tissue effects were observed between both articles at termination 8, 26 and 52 weeks following implantation. Histologically, at 8, 26 and 52 weeks following implantation, the test article was not locally irritant compared with the control article. In terms of performance, the test group yielded significantly higher values of bone to implant contact percentage compared with the control group with the cylindrical or conical defects. No significant test or control article degradation was observed at the level of cylindrical defects until 52 weeks.

The test and the control groups at 52 weeks showed qualitatively similar osseous apposition extent. Lamellar bone was observed directly around the article testifying to a bone remodeling process of a marked grade, demonstrating good marginal defect osteointegration (Figure 3).

In conclusion, following 8, 26 and 52 weeks and under the experimental conditions of the study, the test and control articles showed good intraosseous local tissue effects, including osteointegration along the margin of the defect. The overall performance showed satisfactory bone healing characteristics around the two articles but with a better performance in favor of the test article at the three healing times.

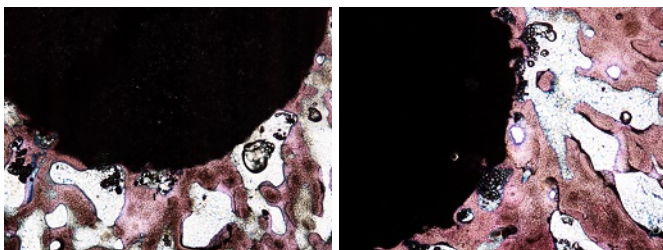


Figure 3: Representative photomicrograph showing local tissue response and bone healing (osteointegration) of the control (left) and test (right) articles at 52 weeks in a femoral model (2X magnification).

## DISCUSSION

The ideal material used for a bone substitute would be 1) radiopaque, 2) low rates of infections, 3) non-conductive of heat or cold, 4) able meet the clinically necessary

biomechanical needs, 5) malleable to fit defects with complete closure, 6) cost efficient, and 7) ready to use.<sup>5</sup>

Calcium phosphate based, hydroxyapatite bone substitutes meet the aforementioned criteria for such a cranioplasty material. Recent reports have also shown the added clinical benefits of the predicate Stryker bone substitute to have reduced rates of cerebrospinal fluid (CSF) leakage and minimal rates of infection compared to alternative cranioplasty materials, such as mesh.<sup>6-8</sup>

DirectInject<sup>®</sup> and its precursor HydroSet<sup>®</sup> are seen by the host immune system as natural biomaterials that closely resemble the mineral phase of bone. These natural biomaterials typically undergo gradual, benign incorporation into the host skeletal structure through cell mediated remodeling and integration. Foreign body reaction or fibrous encapsulation of the implant material was not observed during any of the preclinical animal studies.

DirectInject<sup>®</sup> is the next generation in the evolution of cranioplasty biomaterials. It is a self-setting, calcium phosphate cement intended to repair neurosurgical burr holes, contiguous craniotomy cuts and other cranial defects not intrinsic to the stability of the bony structure. DirectInject<sup>®</sup> is provided in a dual syringe delivery system which eliminates manual mixing steps and subsequently reduces procedural time and costs.

## CONCLUSION

Results of the performance animal testing demonstrate that DirectInject<sup>®</sup> has met all acceptance criteria for biocompatibility. Therefore, the results of the preclinical (animal) tests demonstrate that DirectInject<sup>®</sup> performs as safely and effectively as the legally marketed predicate device (HydroSet<sup>®</sup>).

## REFERENCES

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