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**A preliminary report of percutaneous craniofacial osteoplasty
in a rat calvarium.**

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Abstract

Objective: To evaluate the potential for injectable, permanent bone augmentation by assessing the biocompatibility and bioactivity of subperiosteal hydroxylapatite (Radiesse) deposition in a rat model.

Methods: Fourteen adult Sprague Dawley rats were injected in the parietal skull with 0.2 ml of hydroxylapatite (10 animals) or a carrier gel control (4 animals), using a subperiosteal injection technique on the right and a subcutaneous injection technique on the left. At 1, 3, and 6 months, 3 rats (1 negative control, 2 variables) were sacrificed and calvaria were harvested. At 12 months, the remaining 5 rats were sacrificed. After each

harvest, the specimens were processed and then examined under both light and polarized microscopy for new bone growth at the injection sites.

Results: The inflammatory response was limited with both hydroxylapatite and carrier injections. Injectables were still present 12 months after the injection. New bone formation was only seen when the injection was located deep to a disrupted periosteum. The odd of new bone formation was 48.949 times higher (95% CI (2.637, 3759.961), $p = 0.002$) with subperiosteal hydroxylapatite injections compared to all other combinations of injection plane and injectable.

Conclusion: This preliminary report of subperiosteal hydroxylapatite (Radiesse) injection in a rat model has verified the biocompatibility of injectable hydroxylapatite at the bony interface and suggests the potential for new bone formation.

Introduction

Craniofacial bony reconstruction can be broadly classified as either organic or alloplastic. While autografts remain the gold standard, alloplastic bone substitutes have been widely employed in order to eliminate donor site morbidity, reduce operative time, and facilitate contouring. Presently, calcium phosphate cements (CPC), such as hydroxylapatite (HA), are commonly used for the alloplastic repair of skull defects. Favorable characteristics of CPC include customizability, isothermic setting, biocompatibility, and bioactivity (resorption is countered by new bone replacement).¹

Because of the chemical properties pertaining to setting, open exposure is required

to use CPC effectively, and thus the application of CPC is reserved for large defects, such as those that result from tumor extirpation or extensive trauma. However, facial plastic surgeons are often faced with smaller craniofacial deformities that are of aesthetic concern to the patient but do not warrant the morbidity of open surgery. Examples would include relatively minor traumatic bony injury and contour deficiencies from prior surgery. With these defects in mind, the present study was designed to examine the biologic characteristics of injectable HA (Radiesse, Bioform Medical, Inc., San Mateo, CA, USA) when interfaced with bone. We hypothesized that the biocompatibility and bioactivity of subperiosteal HA injections would mirror those of the cement formulation, making Radiesse potentially useful for the office-based correction of small craniofacial deformities.

Methods

At the outset of this study, approval was obtained from the Institutional Animal Care and Use Committee of Thomas Jefferson University. Fourteen adult Sprague Dawley rats were first anesthetized with isoflurane via a mask apparatus and then injected in the parietal skull with 0.2 ml of HA (10 animals) or a carrier gel control (4 animals). In each rat, the left sided injection was performed just medial to the auricle with a 23-gauge needle in a subcutaneous plane. On the right side of the calvarium, a 20-gauge needle was first employed to elevate the periosteum, and then a 23-gauge needle was used to inject the material directly on to the underlying bone

(again just medial to the auricle). Appropriate placement of the injection was reaffirmed grossly by mobilizing the scalp- the subcutaneous deposits were noted to move with the scalp, while the subperiosteal deposits remained fixed.

Animals were subsequently sacrificed at four time points (1,3,6, and 12 months after the initial injections) and calvaria were harvested for histologic analysis. Each of the first 3 harvests included 2 rats from the HA group and 1 negative control from the carrier gel group. The 12-month harvest again included 1 negative control as well as the remaining 4 rats from the HA group. All of the harvested calvaria were shaved, placed in 10% formalyn, and turned over to the Thomas Jefferson University Department of Pathology.

Using a Mar-Med diamond band bone saw , a coronal section of each calvarium was isolated using the auricles as landmarks. These coronal sections were then sliced further in the sagittal plane to create 5 blocks of tissue, approximately 2-3 mm in thickness, numbered 1-5 while moving right to left. In preparation for microscopic analysis, the tissue sections were decalcified (using CalEx solution) over a 2-day period for a total of 12 hours and then pressed and embedded in paraffin. Finally, each block was cut with a Leica microtome to create 5-micron specimens.

All specimens were stained with hematoxylin and eosin. Under low power magnification, the injectables were located and the surrounding tissue was examined. Proper identification of the injectables was confirmed by examining and comparing separate samples of the HA and carrier gel ex vivo (Figure 1). Polarized microscopy was used to distinguish new (woven) bone from mature (lamellar) bone.

Histologic findings were categorized into data tables that were then analyzed statistically in consultation with the Thomas Jefferson University Department of Biostatistics. Odds ratios, p values and 95% confidence intervals (CI) were calculated using Fisher's conditional maximum likelihood estimation. P values < 0.05 were considered significant.

Results

Histologic data are summarized in Table 1. The inflammatory response to both the HA and the carrier gel control was scant at all time points, regardless of the location of the injection. While multinucleated giant cells were often present (Figure 2), only minimal fibrosis was noted in the specimens. As intended, all of the subcutaneous injections were noted to be above an intact periosteum on histologic review. Seven (2 carrier, 5 HA) out of 13 "subperiosteal" injections were found to be deep to a disrupted periosteum, while the remaining 6 were noted to be in the subcutaneous layer with an intact periosteum beneath. Of note, HA spherules could not be found at the subcutaneous site for specimen 12 and at both injection sites for specimen 13.

Reactive bone was not seen in the absence of periosteal disruption. In 1 of 2 rats with successful subperiosteal carrier injections, reactive bone was present at the time of harvest. This rat, specimen 7, was sacrificed at 6 months (Figure 3). Reactive bone was observed with subperiosteal HA injections in 4 out of 5 rats- specimen 2 from the 1 month harvest (Figure 4), specimen 5 from the 3 month harvest, and specimens 12 and 14 from the 12 month harvest. Interestingly, mature lamellar bone

was seen above the HA spherules in specimen 12, indicating osteointegration. (Figure 5)

In an attempt to analyze the effect of the injection plane (subcutaneous vs subperiosteal) and the injectable (carrier vs HA) on new bone formation, histologic data was re-organized as depicted in Table 2. The primary outcome measure of new bone formation is presented in binary format for all 4 combinations of injection plane and injectable. Of note, injections that were intended to be subperiosteal but were found to be subcutaneous on histologic review were considered “subcutaneous” (n = 6) for the purposes of statistical analysis. In doing so, we assumed that two subcutaneous injections in one rat were independent of one another. Furthermore, any specimen without an identifiable injectable (subcutaneous injection site in specimen 12 and both sites in specimen 13) were excluded. Therefore, out of 28 possible injections (subcutaneous and subperiosteal sites in 14 rats), 25 were considered for the analysis (18 subcutaneous sites, 7 subperiosteal sites).

The odd of new bone formation in the subperiosteal HA injection group was 48.949 times higher than the other 3 combinations in aggregate (95% CI (2.637, 3759.961), $p = 0.002$). The marginal effect of subperiosteal injection was also significant, but a discrete odds ratio could not be computed due to the zero-count cells in the subcutaneous groups (95% CI (4.068, infinity), $p < 0.001$). The marginal effect of HA, however, was not significant. The odd of new bone formation with HA injection regardless of injection plane was only 2.095 times higher than that of the carrier injections (95% CI (0.161, 120.910), $p = 1.000$).

Discussion

Minor deformities of the craniofacial skeleton can be quite bothersome aesthetically to patients. Unfortunately, the morbidity of surgical correction often deters patients from seeking treatment. Radiesse provides an intriguing option for these patients as its main biologic constituent, HA, has been used for over 2 decades in other formulations (initially cermaic, more recently cement) for open craniofacial reconstruction.² It consists of 30% calcium HA microspheres (25-45 μ m) identical to the mineral portion of bone in a carrier gel (1.3% sodium carboxymethylcellulose, 6.4% glycerin, and 36.6% sterile water) that provides cohesiveness.

FDA-approved for the treatment of HIV-related lipoatrophy and moderate to deep nasolabial folds, Radiesse is well-established in facial plastic surgery for soft tissue augmentation.^{3,4} Over the years, various studies have confirmed its safety, longevity and bioactivity (specifically the stimulation of new collagen deposition) when injected subcutaneously.⁵⁻⁷ Not surprisingly, off label uses of Radiesse have arisen as well. In 2007, Stupak et al described HA injection for the treatment of post-rhinoplasty contour deficiencies.⁸ Then, in 2008, after initially experimenting with Radiesse for post-enucleation orbital augmentation⁹, Vagefi et al introduced the concept of injectable osteoplasty for small frontal bone defects.¹⁰ In their novel description, 3 patients were injected with Radiesse in conjunction with other eyelid procedures. Subjective improvement was noted up to 7 months post-op.

To our knowledge, no one to date has examined the histologic effects of Radiesse injection at the bony interface. In this study, we have shown that Radiesse is biocompatible subperiosteally. The inflammatory response to both the HA and carrier

gel was minimal. Furthermore, the HA appears long-lasting, as spherules were identified up to 12 months post-injection. The absence of HA spherules at the subcutaneous site for specimen 12 and at both injection sites for specimen 13 could be indicative of resorption, but may also be explained by extrusion secondary to an error in the injection technique. Osteoactivity was seen in at least one specimen at all time points, indicating that new bone formation can occur as early as 1 month after injection. Based upon this observation, the timing of sacrifice was disregarded during statistical analysis.

In designing the study, we did consider the fact that the trauma of periosteal disruption could trigger osteoactivity and therefore confound results. We attempted to control for this with the carrier only injections, hypothesizing that new bone formation would be either absent or less pronounced without HA. Unfortunately, our technique for periosteal disruption was only successful 54% of the time. Consequently, the numbers for truly subperiosteal HA and carrier injections were simply too low to demonstrate a statistically significant rate of new bone formation between the two. Notably, though, the odd of new bone formation in the subperiosteal HA injection group was significantly higher than the aggregate of all other combinations of injection plane and injectable. We were also able to show that the plane of injection seems to be critical in any effort to induce osteoactivity as none of the subcutaneous injections resulted in new bone formation, regardless of the injectable used. Lastly, our observations suggest that, irrespective of the mechanism that triggered new bone formation, injectable HA can be osteointegrated, as was seen with specimen 12.

This preliminary report of subperiosteal HA (Radiesse) injection in a rat model has verified the biocompatibility of injectable HA at the bony interface and suggested the potential for bioactivity, specifically new bone formation. Refinements in the technique for subperiosteal injection are clearly necessary, and further study on a larger scale is warranted to better elucidate the stimulus for the osteoactivity we observed histologically. Once refined, the potential for permanent bony augmentation via transcutaneous subperiosteal injections may help minimize the morbidity of treating certain craniofacial defects.

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Legends

Table 1. Summary of binary histologic data. The presence of periosteal disruption and new bone formation is indicated with a (+). HA = hydroxylapatite (Radiesse)

Table 2. Summary of binary data for rate of new bone formation, grouped by all combinations of injection plane and injectable. SC = subcutaneous, SP = subperiosteal, HA = hydroxylapatite (Radiesse).

Figure 1. A. amorphous carrier gel and B. hydroxylapatite spherules shown under high power magnification

Figure 2. A multinucleated giant cell is depicted in the center of this high power magnification view of a 1-month hydroxylapatite injection. Note the absence of fibrosis

Figure 3. Under low power, subperiosteal carrier is noted to be embedded in new, woven bone in this 6-month specimen

Figure 4. Under high power, new bone formation is seen amidst subperiosteal hydroxylapatite spherules in this 1-month specimen. Note the disorganized appearance of the new, woven bone as compared to the mature lamellar bone beneath

Figure 5. Under high power, hydroxylapatite spherules from this 12-month specimen appear osteointegrated, with mature, lamellar bone present both below and above the injection

Table 1

Group	Specimen #	Injectable	Subcutaneous Site		Subperiosteal Site	
			Periosteal Disruption	New Bone Formation	Periosteal Disruption	New Bone Formation
1 mo	1	carrier	-	-	-	-
	2	HA	-	-	+	+
	3	HA	-	-	-	-
3 mo	4	carrier	-	-	+	-
	5	HA	-	-	+	+
	6	HA	-	-	+	-
6 mo	7	carrier	-	-	+	+
	8	HA	-	-	-	-
	9	HA	-	-	-	-

12 mo	10	carrier	-	-	-	-
	11	HA	-	-	-	-
	12	HA	HA not identified		+	+
	13	HA	HA not identified			
	14	HA	-	-	+	+
Total Positives			0	0	7	5

Table 2

New Bone Formation			
Injection Plane	Injectable	Response	n
SC	carrier	+	0
		-	6
SC	HA	+	0
		-	12
SP	carrier	+	1
		-	1
SP	HA	+	4
		-	1
			Total n = 25

Figure 1

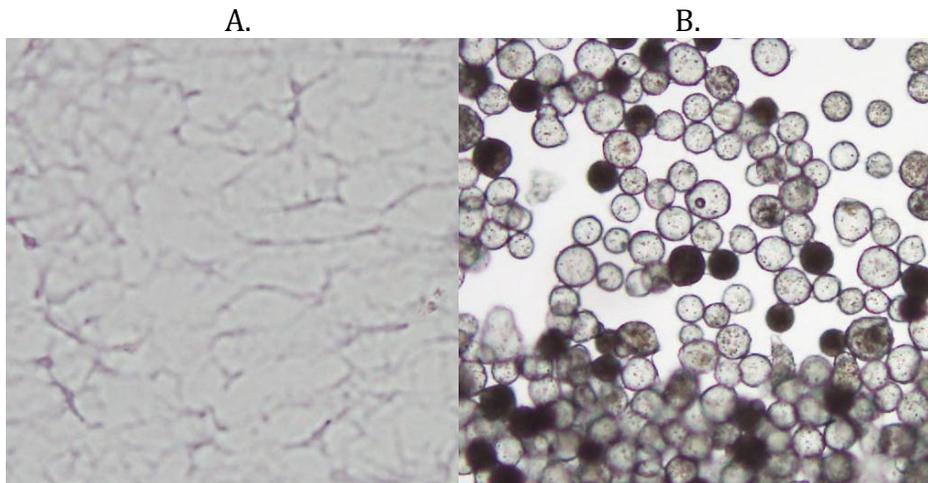


Figure 2

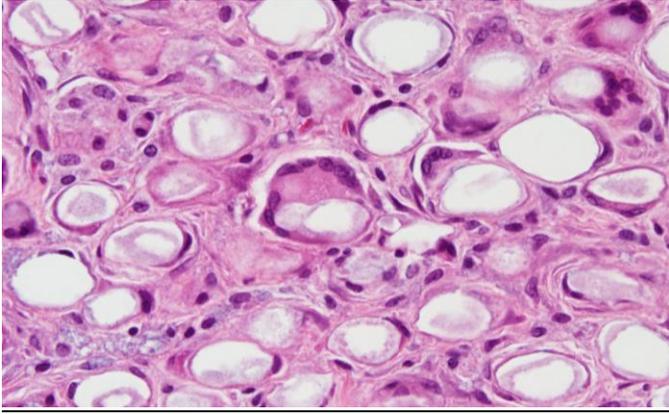


Figure 3

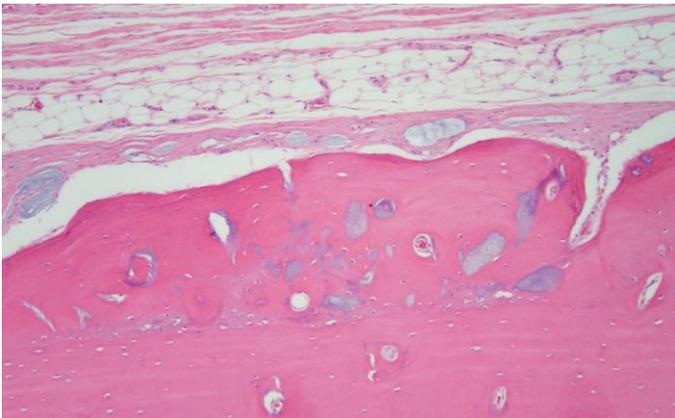


Figure 4

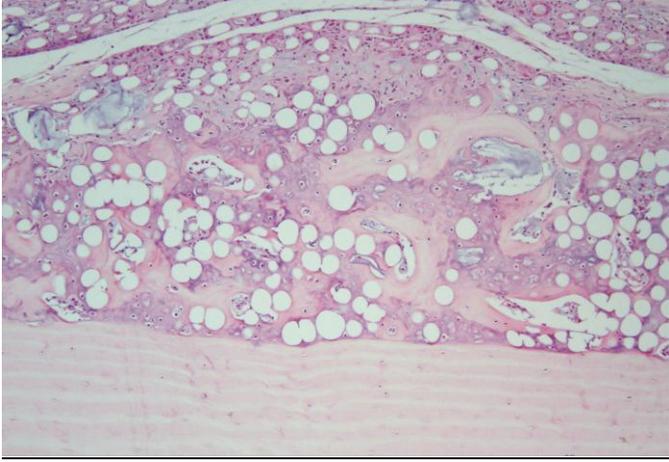


Figure 5

