6-14-2013

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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 hydroxylapatite (n=10) or a carrier gel control (n=4), 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. At 12 months, the remaining 5 rats were sacrificed. After each harvest, the calvaria were examined under both light and polarized microscopy.

Results: The inflammatory response was limited in all specimens. 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.

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INTRODUCTION

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 when interfaced with bone.

METHODS

Fourteen adult Sprague Dawley rats were injected in the parietal skull with 0.2 ml of HA (n=10) or a carrier gel control (n=4). 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).

Animals were subsequently sacrificed at 4 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 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.

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. While multinucleated giant cells were often present (Figure 2), only minimal fibrosis was noted in the specimens. 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)

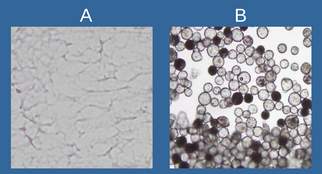

Figure 1. Amorphous carrier gel (A) and hydroxylapatite spherules (B) shown under high power magnification

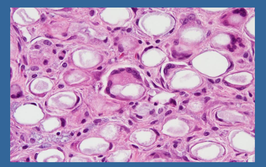

Figure 2. A multinucleated giant cell is depicted in this high power magnification view of a 1-mo hydroxylapatite injection

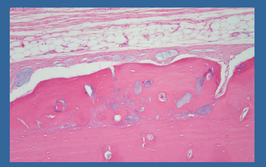

Figure 3. Under low power, subperiosteal carrier is noted to be embedded in new, woven bone in this 6-mo specimen

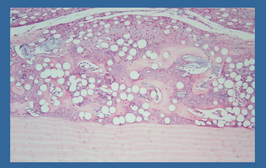

Figure 4. Under high power, new bone formation is seen amidst suboeriosteal hydroxylapatite spherules in this 1-mo specimen

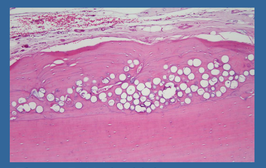

Figure 5. Under high power, hydroxylapatite spherules from this 12-mo specimen appear osteointegrated

RESULTS

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. 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. Furthermore, any specimen without an identifiable injectable (subcutaneous injection site in specimen 12 and both sites in specimen 13) were excluded.

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.

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

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

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)

DISCUSSION

Minor deformities of the craniofacial skeleton can be quite bothersome aesthetically to patients. Radiesse provides an intriguing option for these patients as its main biologic constituent, HA, has been used for over 2 decades in other formulations for open craniofacial reconstruction.[2] 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.[8-10]

To our knowledge, no one to date has examined the histologic effects of Radiesse injection at the bony interface. We have now shown that Radiesse is biocompatible and long-lasting subperiosteally. 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 difference in the 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. Lastly, our observations suggest that, regardless of the mechanism triggering new bone formation, injectable HA can be osteointegrated.

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.

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