Osteogenesis of Osteoblast Seeded Polyurethane-Hydroxyapatite Scaffolds in Nude Mice

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Summary: Biodegradable porous polyurethane (PU) scaffolds were used in a tissue engineering approach to create new bone. Two groups of elastomeric bioresorbable PU disks were seeded with osteoblasts and implanted into nude mice. One group had disks of pure PU while the other group had disks of PU- hydroxyapatite composite (PU-HA). After 5 weeks both groups showed radiographic and histologic evidence of significant bone formation. As the new bone formed it replaced the PU scaffolds. Although not statistically significant, there was a trend toward more bone formation in the PU-HA group. Bioresorbable PU shows promise for use in bone tissue engineering.

Keywords: bone; hydroxyapatite; polyurethanes; tissue engineering; scaffold

Introduction

It is generally believed that tissue engineering technology has great potential to fill the needs for bone regeneration, however the ''ideal'' scaffold for bone tissue engineering has yet to be identified. Most researchers agree that it should be made of a bioresorbable material with interconnected pores to allow tissue ingrowth and free diffusion of nutrients. In addition, it should be biocompatible, biomechanically appropriate for the host tissue, and preferably resorbe as osseous regeneration proceeds.

Segmented polyurethane (PU) is one of the most biocompatible materials known today and possesses many of the characteristics of the ideal bone scaffold. For over two decades polyurethanes (PU) have been used in a variety of medical devices such as intravenous catheters, vascular grafts, cartilage replacements, artificial hearts, and pacemaker lead insulation.[1–3] By adjusting the various processing techniques the biologic and mechanical properties of the scaffold can be extensively modified to match that of the host tissue. Polyurethanes can also be seeded with osteogenic growth factors and cells to enhance healing.[4]

Previous in vitro studies have shown polyurethane can support the growth of osteoblast precursor cells and allow the deposition of calcium phosphate.[5] Another study has shown degradable polyurethane can induce bone regeneration in critical-sized iliac defects in healthy and in estrogen deficient sheep.[6,7] Our hypothesis was that osteoblast seeded porous polyurethane foam can act as a scaffold for in vivo osteogenesis. We tested this hypothesis by seeding bovine osteoblasts onto degradable PU and PU-nano hydroxyapatite (PU-HA) scaffolds and implanting them into the subcutaneous tissue of nude mice. This study is the first step in determining if the cell-seeded PU scaffolds hold promise for bone tissue engineering and whether the

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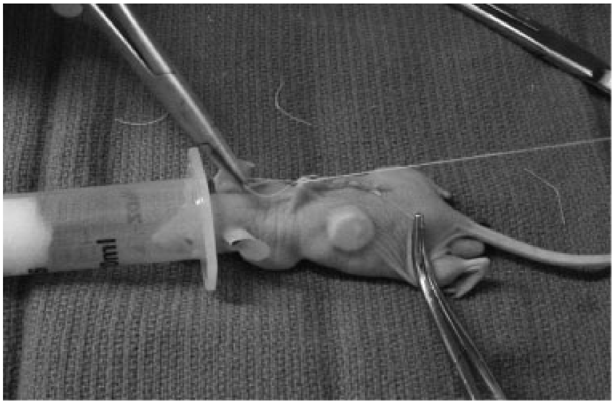

Figure 1.
Biodegradable polyurethane scaffold implanted into the subcutaneous space of a nude mouse.

addition of nano-HA can facilitate better bone formation.

Materials and Methods

The animal model was approved by the IACUC at the Medical University of South Carolina. Stored primary fetal bovine osteoblasts were propagated in monolayer culture until confluence. The harvested cells were then loaded onto bioabsorbable PU and PU-HA scaffolds at a concentration of at 5×10^6 cells/scaffold. Each circular implant measured 10 mm diameter and 2.5 mm thick with a porosity of 90% and interconnected pore size of $360\mu, \pm 120\mu$.

Fourteen 4–6 week old male, athymic mice were obtained and divided into two equal groups. Under general anesthesia and sterile conditions a 10mm dorsal midline incision was made and one cell seeded PU scaffold ($n = 7$) or one cell seeded PU-HA scaffold ($n = 7$) was implanted into the dorsal subcutaneous space of the mice. Incisions were closed with 5-0 vicryl suture and each mouse was allowed unrestricted activity in his cage. (Figure 1)

After 5 weeks each mouse was sacrificed and implants were harvested and radiographed using a high-resolution Faxitron unit. Each explant was then processed for histologic analysis; 5μm sections were made and stained with Hematoxylin and Eosin. Each section was photographed and saved as a digital image. The percentage of new bone formation was quantified with a color- based histogram method utilizing

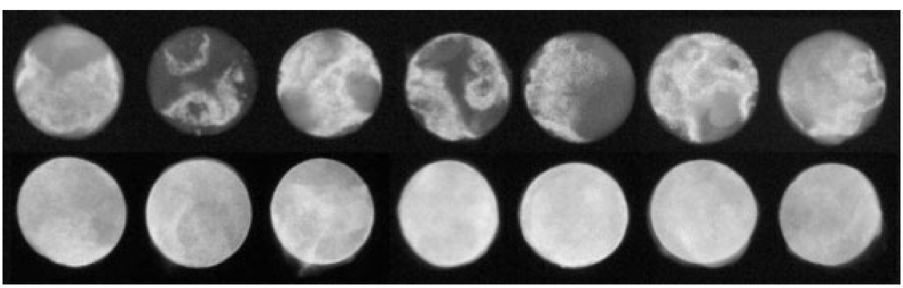

Figure 2.
Radiograph showing bone formation (white areas) in both PU only implants (top row) and the PU-HA implants (bottom row). The more uniform radio-opacity of the bottom row is likely due to the nano hydroxyapatite in the implants.

Figure 3.
Histology: Significant bone formation in both PU only implants, (top row) and the PU-HA implants(bottom row). The lighter areas are PU scaffold and the darker areas are new bone.

Photoshop software.[8] The amount of bone formation was compared using unpaired student t test, significance was set at $P < 0.05$.

Results

Both the radiographs and histological sections showed significant bone formation in both groups (Figure 2 and 3). The amount of new bone formation in each implant, expressed as a percent of the total area of the implant, is shown in Table 1. The PU group, on average, had 47% of the scaffold replaced by bone over the 5 week period. The PU-HA group averaged 56% over the same period, although this difference was not statistically significant.

Table 1.
Percentage of new bone in each specimen quantified from digital images similar to that in Figure 3.

PU only	PU-HA
55	50
35	86
35	41
74	62
31	63
43	35
56	59
Mean 47.0	56.6
SD 15.5	16.8
P = 0.26.	

Discussion

Within 5 weeks of implantation much of the polyurethane material was resorbed and only about half of the remaining scaffold could be seen on the histologic sections. All implants showed areas of rich woven bone and most areas on the stained sections were populated with cells. It is interesting to note the presence of cartilaginous cells in many of the sections, which may indicate a process of endochondral ossification taking place. Although the difference in bone formation between the two scaffolds is not statistically significant we believe there may be a trend toward greater bone formation in the PU-HA group. Additional testing with larger numbers of specimens would be needed to confirm this.

Conclusion

In summary, the two polyurethane scaffolds facilitated bone formation when seeded with bovine osteoblasts, showing promise for future applications in bone tissue engineering. Further studies are planned to evaluate osteogenesis at different time periods in nude mice and also to evaluate bone defect filling using larger animal models.

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