https://doi.org/10.48047/AFJBS.6.12.2024.3472-3477

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

BIOCOMPATIBILITY AND INFLAMMATORY RESPONSE OF COMMERCIAL BONE GRAFT SUBSTITUTES: AN *IN-VIVO* MURINE AIR POUCH STUDY

Dr. Sairamakrishnan S*

Assistant Professor, Department of Orthopaedics,
Sri Lakshmi Narayana Institute of Medical Sciences & Hospital,
Osudu, Puducherry - 605502

Article History
Volume 6, Issue 12, 2024
Received: 02 Jun 2024
Accepted: 25 Jun 2024
doi:
10.48047/AFJBS.6.12.2024.3472-3477

Abstract:

This study aims to assess the biocompatibility and inflammatory response of four commercially available bone graft substitutes using an in vivo murine air pouch model. The substitutes included coralline hydroxyapatite in its pure form, known for its osteoconductive properties, and three other products with both osteoconductive and osteoinductive properties. Over a 14-day implant period, samples were collected from the pouches to extract mRNA markers for inflammation and to analyze cellular responses through various histological characteristics. The findings indicate that while autogenous bone grafts are preferred due to reduced morbidity risks, bone substitutes can be effective yet raise safety concerns. Hematology and polymerase chain reaction analyses revealed that high demineralized bone matrix to carrier ratios correlate with elevated inflammatory responses. Coralline hydroxyapatite showed relatively mild inflammation compared to other substitutes. These results underscore the importance of considering both efficacy and safety when selecting bone grafting methods. Further studies are necessary to enhance treatment outcomes and minimize morbidity.

Keywords: Biocompatibility, bone graft substitutes, inflammation, osteoconductive, osteoinductive.

Introduction

There is a wide range of subspecialties and anatomic regions where bone grafting can be used in orthopedic surgery [1-3]. Patients can receive autografts or bone from cadavers. Despite this, both sources have problems. Donor-site complications can occur during autograft bone harvesting. The possibility of inflammatory or immune reactions also exists with allograft bone. For the majority of cases, autografts remain the gold standard of bone grafting despite recent technological advances, although alternatives may be available that are less morbid. [4,5] A decade ago, there were no alternatives to bone grafts. It might be possible to replace conventional bone grafting techniques with these substitutes if they prove safe and effective.

DBM implanted in 1965 was the first to replace bone grafts. In the decades since urist developed osteoconductive, osteo-inductive, and osteogenic materials for bone grafts, researchers have studied these materials extensively [6-9]. These criteria must be met by providing either bone precursors or bone cells, triggering bone formation through biochemical signaling, and providing a physical scaffold for bone formation [10, 11]. All three properties must be present in a bone graft for it to function. A variety of factors are involved in the formation of bone in fresh autografts, such as proliferation of cells, signaling molecules, and structural changes in molecules. These qualities are now possible in engineered bone graft substitutes. The osteoconductive and osteo- inductive properties of DBM have been demonstrated in studies. As well as being a viable alternative to bone grafts, DBM is shown to promote bone growth. [12-16] However, there is limited or no data on its efficacy and safety, especially in regard to its immunogenicity. There is little immunological evidence linking DBM products to nephrotoxicity, despite some having been shown to cause it in vivo. The safety and effectiveness of the product are still under investigation, as well as the most effective formulation and preparation methods.

Materials and methods

An injection of 3 ml of air subcutaneously along with a 25-gauge needle was used for biocompatibility testing on female balb/c MICE. [17-19] The first injection was 3 ml of air subcutaneously along with a 25-gauge needle. During the next five days, the air pouches received an additional 1ml of air every other day. Pouch punctures and the insertion of test material started the study on the sixth day. An 8–0 Prolene suture was used for closure of the pouches. The anti-infective solution was injected using 25-gauge needles. In each group, six mice were randomly assigned. A sterile solution of phosphate buffered saline was injected into the first group of pouches. Implants of sterile ProOsteon® were applied to the second group (Interpore Cross, Irvine, CA). Hydroxyapatite is osteoconductive due to its porosity. Because DBX is based on sodium hyaluronate, it contains approximately 32% DBM. With Accell Connexus, 70% of DBM is encapsulated in a proprietary reverse-phase medium, as opposed to Accell DBM-100. A 14-day period was spent implanting the mice in CO2 chambers and sacrificing them. In this study, subcutaneous tissues were dissected without using pouches. Nucleic acids are extracted by freezing the pouches after they have been cut in half and formalin has been applied. Paraffin blocks were embedded in paraffin-fixed and dehydrated tissue samples. The sample was carefully positioned on the mouse while maintaining its original shape and orientation. Hematoxylin and eosin stainings were used to determine the presence of cellular infiltrates within pouches and pouch membranes. For each specimen, an imaging analysis program from Media Cybernetics (Bethesda, MD) was used to examine four sections in a minimum. The thickness of each pouch was measured evenly at six points around its circumference. It was also possible to count nucleated cells inside the pouch cavity and throughout the pouch membrane thickness. By dividing the number of cells by a mm2 area, we determined the cellular density based on previous descriptions. Nuclear aspect ratio is used in image analysis to distinguish mononuclear from fibroblastic cell morphology. Macrophages and fibroblasts were also visually identified and counted in order to verify the data from the image analysis.

The presence of inflammatory cytokines was detected using polymerase chain reaction (PCR). Tel- Test provided instructions for extracting this fluid from Friendswood, TX. RNA extracts were diluted with PCR buffer, MgCl2, nucleotide triphosphates, RNase inhibitors, and reverse transcriptase (Perkin-Elmer Cetus, Norwalk, CT) were added to synthesis buffer as part of cDNA library preparation. Incubation times at 25°C, 48°C, and 95°C were five minutes, 25 minutes at

25°C, and 25 minutes at 48°C. By (Mountain View, California), IL-1 primer sequences have been developed. This experiment used 1.5 liters of MgCl2, two liters of cDNA, two liters of primers, and two liters of PCR buffer. Denaturement was achieved by heating at 94°C for one minute then annealing at 60°C for one minute. We extended denatured RNA and cDNA hybrids at 60°C, 72°C, 94°C and 72°C after denatured RNA and cDNA hybrids were annealed at 60°C. This sequence was repeated 35 times on a Perkin-Elmer Cetus DNA thermal cycler. Dilutions of the amplification solution and loading buffer were used in the analysis on agarose gels. A digital imaging system and an Alpha Innotech system were used to measure the density of the cytokine bands. Normalizing CYTOKINE levels was achieved using GAPDH.

Statistics

Analysis of variance (ANOVA) analysis revealed that pouch membrane thickness, pouch infiltrate density, and cellular infiltrate density in pouch cavities were significantly different from one another. The means of the treatments were compared if there was a significant difference between them. An ANOVA with a single factor was used again to analyze differences between the PCR means. A significant difference between the treatments was determined based on students' t-tests. Statistical significance was determined by P 0.05 for all results.

Results

To determine if inflammation responses to implanted biomaterials are characterized by increases in thickness and density of cellular infiltrate, histological characteristics of the air pouch membrane wall were examined and compared to a saline control. Approximately 83.4 cells per mm^2 were found in the membranes, which had a mean thickness of 47.7 millimeters. A significant difference was found in the thickness of the membranes in Accell, DBX, and ProOsteon bone graft substitutes (P0.05). There were no significant differences between compounds in any of the other mean values. Using a one-factor ANOVA, all compounds and saline control showed similar cellular density within the air pouch membrane walls.

Table 1: Brief summary of RT-PCR and histopathology data

Bone graft substitute	**Membrane thickness (mean ± SE, µm)**	**Pouch density (mean ± SE, cells/mm^2)**	**Macrophages/monocytes: fibroblasts**	**Interleukin-1 expression (mean ± SE, copies)**
Saline	48.8 ± 3.5	2.0 ± 0.4	2:5	40,508 ± 13,072
ProOsteon®	177.8 ± 8.4	40.8 ± 2.3	2:44	114,940 ± 23,606
DBX®	203.4 ± 9.8	74.2 ± 2.0	2:5	158,204 ± 50,086
Accell Connexus®	135.9 ± 7.6	85.0 ± 2.7	2:2.5	157,126 ± 34,348
Acell DBM-100™	145.5 ± 7.6	108.6 ± 3.3	2:4.4	268,148± 43,558

Discussion

Researchers have found that allograft source, preparation method, particle size, and carrier substance influence DBM's composition. It has been suggested by several studies that bone graft substitute preparation procedures may affect the biocompatibility of their substitutes, resulting in adverse effects on patients. There have, however, been few studies that examine how patient outcomes are affected by these variations. In order to remove potentially infectious agents from bone allografts, various treatments have been applied, including washing, antibiotic treatment,

irradiation, and other treatments. Additionally, processing has been shown to inactivate a variety of infectious viruses, including hepatitis B and C viruses and cytomegalovirus, in addition to removing bacterial contaminants. [20-24] Whether the differences in efficacy are due to differences in carrier types or preparations is unclear. Hydroxychloric acid is used to demineralize the tissue and then it is dried. DBM and a carrier are mixed together to alter the final product's texture, viscosity, and other characteristics.

There are a number of factors that affect the efficacy and safety of DBM products. Both animal and human studies have shown that DBM's osteo inductive capacity is significantly influenced by the donor's age. In addition, DBM used in the carriers differs significantly by type and concentration. Several carriers do not carry anything, including glycerol, porcine collagen, hyaluronic acid, lecithin, calcium sulfate, etc. Most DBM products contain around 20% to 40% DBM. DBM products have been infused with additional delivery systems to alter their physical properties in addition to their carrier substance, which has been investigated in some studies. Various materials and techniques are used to manufacture similar products that are intended for the same purpose, so it is unclear what should constitute an ideal bone graft replacement composition. Commercially available bone graft substitutes were evaluated for their inflammatory properties. Murine air pouches were used to study hydroxyapatite and demineralized bone graft compounds. The inflammatory responses to all products were significantly higher than those to saline. As a substitute for DBM, sodium hydroxyapatite was used to reduce inflammation. With increasing DBM concentrations, inflammation increased in DBM products tested. In order to understand how this relationship works, it is important to distinguish between DBM products that have different ratios of DBM to carrier. Several commercially available DBM products were compared using a spinal fusion model, according a study [25]. Osteo inductivity and fusion rates were determined by histological sections. Athymic mice were tested for osteo inductivity using an immune incompetent mouse model.

Two shortcomings have been identified in our model of assessing inflammatory reactions induced by bone graft substitutes. A model based on animals was used as a starting point. Using human biology as an analogy should be done with caution. A species-specific analysis of biomaterials is necessary. Due to the fact that DBM products are derived from human tissue, it is theoretically possible for them to elicit a xenograft reaction. In addition, these materials were used in this study to assess the inflammatory response induced in the murine inflammatory air pouch. It was not claimed that they were effective. The data presented here should be used with caution when determining the ideal bone graft substitute. Further research is needed into the formulations of bone graft substitutes. This study could be followed up with an investigation of additional bone graft alternatives. By implanting bone graft substitute materials within the air pouch, bone induction and production can be quantified. Ideally, prospective clinical trials comparing these products would be conducted. The biology of bone graft substitutes has been gained through studies such as ours, but more research is necessary.

Conclusion

There was a significant inflammatory response to all products tested in comparison with saline control. Inflammation was less widespread with coralline hydroxyapatite than with DBM. Within the groups, inflammation increased as DBM concentration increased. Our study suggests that clinical biocompatibility is influenced by DBM proportions.

References

1. Finkemeier CG. Bone-grafting and bone-graft substitutes. J Bone Joint Surg Am. 2002;84- A:454–464.
2. Iwata H, Sakano S, Itoh T, Bauer TW. Demineralized bone matrix and native bone morphogenetic protein in orthopaedic surgery. Clin Orthop Relat Res. 2002;395:99–109.

3. Urist MR. Bone: formation by autoinduction. Science. 1965;150:893–899.
4. Bauer TW, Muschler GF. Bone graft materials. An overview of the basic science. Clin Orthop Relat Res. 2000;371:10–27.
5. Bucholz RW. Nonallograft osteoconductive bone graft substitutes. Clin Orthop Relat Res. 2002;395:44–52.
6. Vaccaro AR, Chiba K, Heller JG, et al. Bone grafting alternatives in spinal surgery. Spine J. 2002;2:206–215.
7. Oakes DA, Lee CC, Lieberman JR. An evaluation of human demineralized bone matrices in a rat femoral defect model. Clin Orthop Relat Res. 2003;413:281–290.

8. Einhorn TA, Lane JM, Burstein AH, Kopman CR, Vigorita VJ. The healing of segmental bone defects induced by demineralized bone matrix. A radiographic and biomechanical study. J Bon Joint Surg Am. 1984;66:274–279.

9. Han B, Tang B, Nimni ME. Quantitative and sensitive in vitro assay for osteoinductive activity of demineralized bone matrix. J Orthop Res. 2003;21:648–654.

10. Edwards JT, Diegmann MH, Scarborough NL. Osteoinduction of human demineralized bone: characterization in a rat model. Clin Orthop Relat Res. 1998;357:219–228.

11. An HS, Simpson JM, Glover JM, Stephany J. Comparison between allograft plus demineralized bone matrix versus autograft in anterior cervical fusion. A prospective multicenter study. Spine. 1995;20:2211–2216.

12. Feighan JE, Davy D, Prewett AB, Stevenson S. Induction of bone by a demineralized bone matrix gel: a study in a rat femoral defect model. J Orthop Res. 1995;13:881–891.

13. Ladd AL, Pliam NB. Use of bone-graft substitutes in distal radius fractures. J Am Acad Orthop Surg. 1999;7:279–290.

14. Russell JL, Block JE. Clinical utility of demineralized bone matrix for osseous defects, arthrodesis, and reconstruction: impact of processing techniques and study methodology. Orthopedics. 1999;22:524–531. PubMed] [Google Scholar]

15. Yoon ST, Boden SD. Osteoinductive molecules in orthopaedics: basic science and preclinical studies. Clin Orthop Relat Res. 2002;395:33–43.

16. Bostrom MP, Yang X, Kennan M, Sandhu H, Dicarlo E, Lane JM. An unexpected outcome during testing of commercially available demineralized bone graft materials: how safe are the nonallograft components. Spine. 2001;26:1425–1428.

17. Wang JC, Kanim LE, Nagakawa IS, Yamane BH, Vinters HV, Dawson EG. Dose-dependent toxicity of a commercially available demineralized bone matrix material. Spine. 2001;26:1429– 1435.

18. Seeherman H, Li R, Wozney J. A review of preclinical program development for evaluating injectable carriers for osteogenic factors. J Bone Joint Surg Am. 2003;85-A:96–108.

19. Wooley PH, Morren R, Andary J, et al. Inflammatory responses to orthopaedic biomaterials in the murine air pouch. Biomaterials. 2002;23:517–526.

20. Ottaviani RA, Wooley P, Song Z, Markel DC. Inflammatory and immunological responses to hyaluronan preparations: study of a murine biocompatibility model. J Bone Joint Surg Am. 2007;89:148–157.

21. Maddox E, Zhan M, Mundy GR, Drohan WN, Burgess WH. Optimizing human demineralized bone matrix for clinical application. Tissue Eng. 2000;6:441–448.

22. Takikawa S, Bauer TW, Kambic H, Togawa D. Comparative evaluation of the osteoinductivity of two formulations of human demineralized bone matrix. J Biomed Mater Res A. 2003;65:37–42.

23. Swenson CL, Arnoczky SP. Demineralization for inactivation of infectious retrovirus in systemically infected cortical bone: in vitro and in vivo experimental studies. J Bone Joint Surg Am. 2003;85-A:323–332.

24. Ad Hoc Working Party on Biotechnology/Pharmacy. Validation of virus removal and inactivation procedures. Pharmacol Toxicol. 1991;69:144–148.

25. Pinholt EM, Solheim E. Osteoinductive potential of demineralized rat bone increases with increasing donor age from birth to adulthood. J Craniofac Surg. 1998;9:142–146.