Australian
Biotechnologies
Life Enhancing Allografts

Allovance® Osteoinductive Technology

Introduction

Autograft is the most widely used bone void filler due to its osteoconductive and osteogenic properties. Osteoconduction is defined as the material's ability to serve as a scaffold for bone formation whilst osteogenesis refers to the process of bone formation. However, autografts have their disadvantages including surgical site morbidity, risk of infections, additional operating time and limited availability[1].

Allografts have been explored as an alternative solution to overcome the drawbacks of autografts. Allografts can be processed to become osteoinductive - the ability to induce non-differentiated cells into osteoblasts that are required to initiate bone formation in an ectopic site. However, all bone processing techniques are not equal, and can result in significant implications to the handling, mechanical, biological and regenerative potential of the bone graft. Importantly, the properties of the graft must work in concert with the surgeon and hardware to achieve the clinical outcome.

Allovance® Osteoinductive allografts are made from human cortical bone manufactured using proprietary processing technologies that have been checked extensively through validation testing to ensure reproducibility and repeatability. Every lot for each product is tested using the 'gold standard' osteoinductive testing as per the ASTM guide for *in vivo* evaluation of osteoinductive potential[2].

Demineralized Bone Allografts

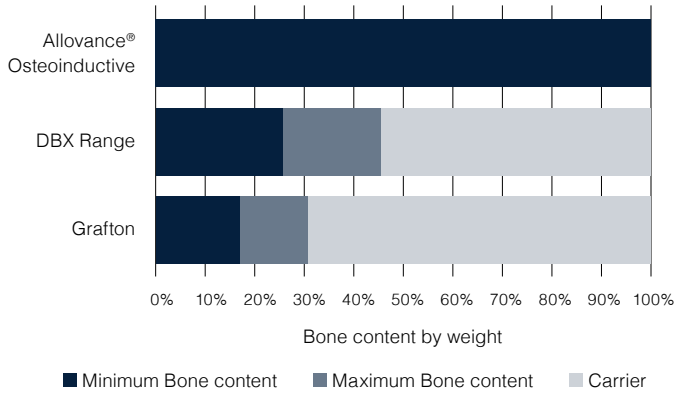
Demineralization is the process of treating bone to remove the inorganic mineral phase whilst preserving the organic phase composed of collagen and non-collagenous proteins that participate in bone formation and remodelling[3].

A wide range of demineralized bone allografts are commercially available with varying size, shape and composition to improve the handling properties[1]. Carriers are frequently added to demineralized bone allografts to facilitate handling and graft containment. However, as these carriers are non-osteoinductive they can elicit an unwanted reaction and risk hindering the bone formation process[4]. Demineralization treatment can also damage the crucial organic components that affect the *in vivo* performance of the resulting demineralized bone matrix and must be carefully controlled to retain the biological potential of the material.

Allovance® Osteoinductive allografts are made from 100% bone and are tested in accordance with the code of Good Manufacturing practice for Blood and Blood Components, Human Tissues and Human Cellular Therapy Products Method of Testing[5]. The processing technologies implemented to produce Allovance® Osteoinductive allografts are designed to preserve the natural collagen structure of the allograft and the osteoinductive proteins (the bone morphogenetic proteins, BMPs.

Comparison of Bone Content

Figure 1: Graph displaying the range in bone content percentage in various products[3]



Osteoinductivity

Demineralized bone allografts have been shown to induce bone formation, with Urist et al.[6] first identifying the process as 'autoinduction'. However, the process of demineralization itself does not guarantee the allograft to be osteoinductive due to a number of factors including manufacturing process, storage/environmental conditions, and donor factors[4].

Therefore, validating the final product as osteoinductive prior to clinical use is essential to prevent the risk of implanting a non-inductive material that would ultimately fail to assist bone formation at a healing site.

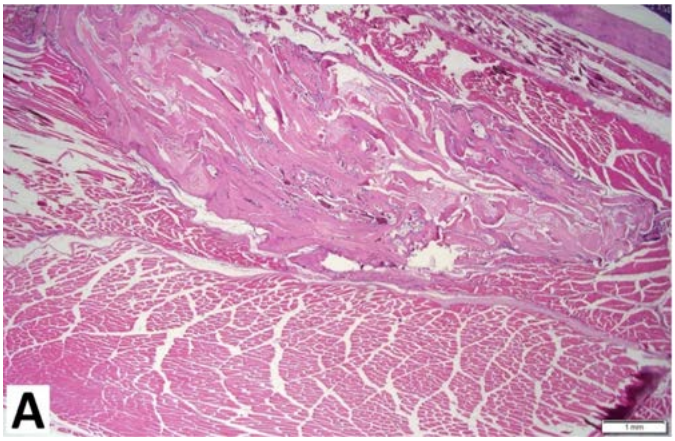
Testing for Osteoinductivity

In vivo testing of ectopic bone formation was originally described by Urist et al.[6] and has since become the gold standard process to assess osteoinductivity under the ASTM International standard for *in vivo* testing[2]. The test consists of implanting grafts into an ectopic pouch of an athymic rat, followed by a histological analysis to identify evidence of new bone formation. By testing in an ectopic environment, the product can be directly accountable for any bone formation, without being subjected to variables from the biochemical and mechanical environment of an orthotopic model that could aid in bone growth.

The use of *in vitro* testing to assess osteoinductivity has been reported to provide a hastened and more economic predictive system. For example, bone morphogenic proteins (BMPs) are growth factors that have been associated with osteoinductivity and can be measured using protein quantification ELISA. C2C12 cell lines have also been used as an indirect quantification of osteoinductivity to measure the alkaline phosphatase (ALP) activity that results from C2C12 cell differentiation into osteoblasts[7]. Although *in-vitro* testing can provide insight into the roles of various growth factors in osteoinductivity, correlations with *in-vivo* screening have been poor. Consequently, *in vitro* testing alone has not been able to provide reliable results to ensure a product will induce bone formation in the desired clinical setting[8,9].

Osteoinductive Verified Products

In Australia a separate Therapeutic Goods Administration approval is required to claim a bone graft is osteoinductive. The product must undergo osteoinductivity testing for each batch of the product and must pass for osteoinductivity claims to be made. Furthermore, osteoinductive stability validations must be performed to ensure that the bone graft remains osteoinductive throughout the shelf life of the product. These validations must be approved by the Therapeutic Goods Administration prior to any claims being made.



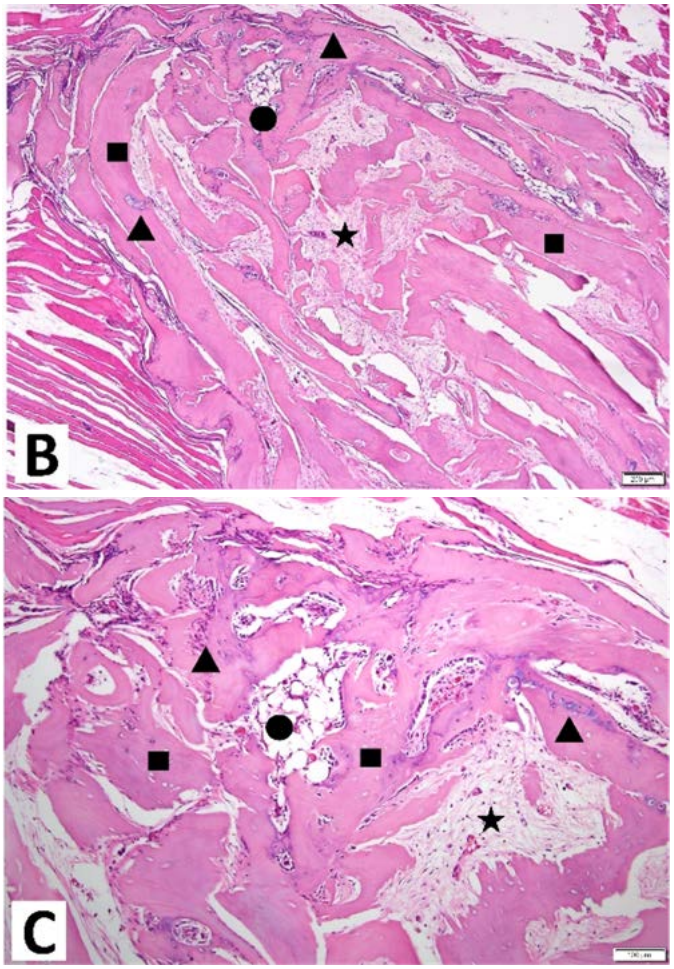


Figure 2: Low magnification overview of in-vivo testing (magnification x1.25 (A), x10 (B) and x20 (C) of paraffin histology of Demineralised Bone Fibres at 4-week time point.

■ indicates implanted demineralised graft

● indicates bone marrow

★ indicates fibrous tissue

▲ indicates chondroblast activity and new bone

Allovance® Osteoinductive Allografts

The Allovance® Osteoinductive product range are osteoinductive certified allografts that are only released if the product batch had passed the *in vivo* testing with evidence of bone formation, as per the Therapeutic Goods Administration requirements.

Osteoinductivity Testing

Every batch of Allovance® Osteoinductive allograft is sent to an independent, Therapeutic Goods Administration accredited laboratory and tested for osteoinductivity as per the required standards[10].

Using the pre-validated *in vivo* osteoinductive model, a sample from each batch is surgically implanted into a pocket created between the adductor brevis and semimembranosus muscles of athymic nude rat models. The implant site is removed after 28 days post implantation, then formalin fixed, decalcified, paraffin embedded and cut into sections. The sections are then stained with hematoxylin and eosin (H&E) in preparation for an extensive histopathological analysis by a trained histologist. Evidence of new bone formation is determined based on the presence of chondroblasts and/or chondrocytes, osteoblasts and/or osteocytes, cartilage, osteoid, new bone and bone marrow.

A certificate stating the results of the test for each batch is then provided by the external laboratory. The Allovance® Osteoinductive Allografts is only released if the certificate confirms the batch has passed the *in-vivo* Osteoinductivity test.

Osteoinductive Stability Studies

The osteoinductive claims of Allovance® Osteoinductive allografts have been verified and validated for the entire period of the product's shelf-life through extensive real-time stability studies, as per the Therapeutic Goods Administration requirements[11-17].

The real-time studies have demonstrated stable osteoinductivity results for the Allovance® Osteoinductive allografts using the gold standard *in vivo* model throughout the duration of the products' shelf-life and will continue to demonstrate osteoinductive stability for longer storage times.

In vivo Osteoinductivity Test

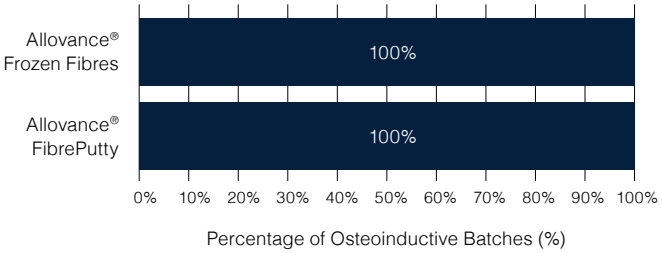


Figure 3: Graph displaying the number of product batches that have passed the *in vivo* Osteoinductivity test as a percentage.

Historic data from routine data collection of over five-years has demonstrated consistent osteoinductivity results for the Allovance® Osteoinductive allografts (See Figure 2). From a sample of 212 frozen demineralized fibres manufactured, 100% of the samples tested had demonstrated the ability to promote bone formation in the *in vivo* model after 28 days from implantation. The historic data demonstrates how the implanted processing techniques can consistently maintain osteoinductive properties in the grafts.

Closing Summary

The Allovance® Osteoinductive allografts are made from 100% bone using proprietary, patent protected processing technologies.

Extensive measures are taken to ensure the released Allovance® Osteoinductive allografts are osteoinductive prior to clinical use through thorough validation and verification of manufacturing processes.

Every batch is tested using the 'gold standard' *in vivo* testing at a Therapeutic Goods Administration accredited laboratory to verify the product is osteoinductive prior to release.

The osteoinductive claim of the Allovance® Osteoinductive allografts have been validated for the entire product shelf-life through extensive real-time stability studies that demonstrated stable osteoinductivity, as per the Therapeutic Goods Administration requirements.

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