# Commercial Hurdles to Stem Cell-Derived Bone Grafts:

## From Lab to Marketplace

Stem Cell Implants

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# Executive Summary

Many tissues, extracellular matrices, and proteins currently sourced from tissue banks or livestock can now be grown in vitro using stem cells. Compared to cadaver bone sourced from a tissue bank, human stem cell-derived bone provides greater lot consistency and no risk of disease transmission. However, considerable hurdles remain before a stem cell-derived bone graft can be brought to the market. Dr. Allan Dovigi of Stem Cell Implants outlines these hurdles below, and offers potential strategies to overcome them.

# Introduction

Bone grafting is a surgical procedure widely used in the dental and orthopedic industries, with approximately 2.2 million performed each year.[1] Bone grafts have many applications. Dental implants, for example, require sufficient bone for support and functionality, and ~70% of dental implant procedures involve bone grafting. Craniofacial reconstruction often requires extensive bone for treatment of trauma, repair of pathologic defects, and other congenital deformities. An additional 500,000 bone grafts are performed annually for spinal fusions and non-union fractures.

Current treatment options for bone grafting are autografts, allografts, or synthetic grafts, of which allografts account for a majority of procedures (~57%).[2] Each approach has drawbacks. Autografts require a donor site to supply bone, which entails a second surgical procedure and associated morbidities. Allografts use bone sourced from tissue banks, which results in lot inconsistencies and the risk of disease transmission. Finally, synthetic substitutes lack osteoinductive properties, and are often mixed with autogenous or allogenic bone to improve their success rate.

Many patients have medical, ethical, or religious concerns about the origin and type of bone graft recommended by their doctor, which can also limit the range of treatment options. Studies show the degree of acceptance or rejection for grafts varies significantly by procedure, with allogenic grafts eliciting the highest refusal rate at 40%, followed by autogenous grafts at 34% and xenografts at 32%.[3] Reasons for patient refusal include fear of pain and discomfort for autogenous grafts, and the risk of disease transmission for allogenic grafts and xenografts. Allogenic grafts present additional concerns about the origin of the bone for many patients, often those with religious affiliation.

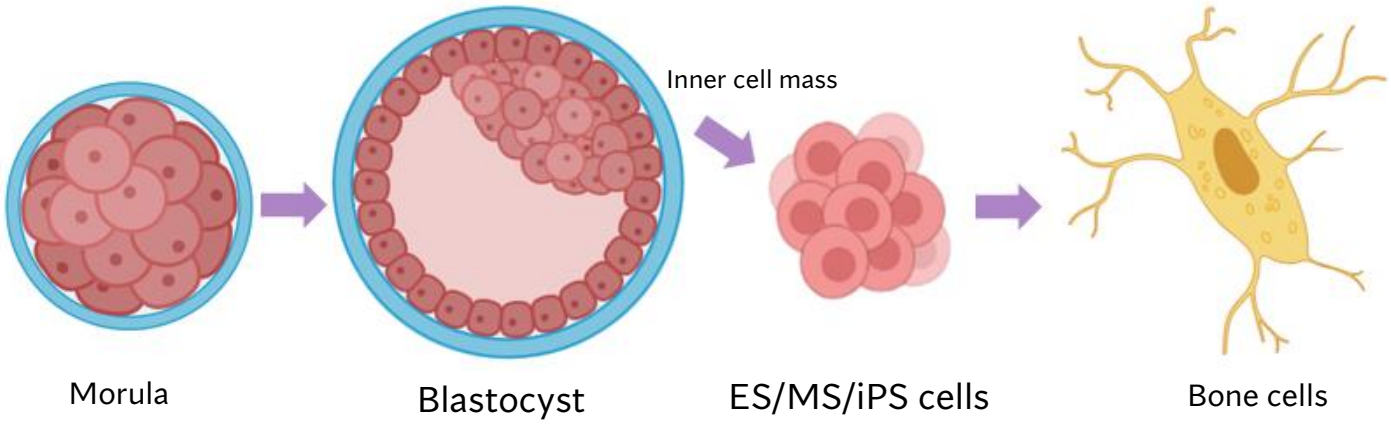
Research has proven the feasibility of growing bone, collagen, and other extracellular matrix (ECM) materials in vitro with stem cells. Stem cells offer unique advantages, including a potentially unlimited supply of bone, greater osteogenic potential, and no risk of cross-infection. However, important hurdles remain before the technology can be brought from the lab to the marketplace.

# Hurdles

## Cell Line

One hurdle to the commercial viability of stem cell-derived bone grafts is the selection of an optimal cell line. In choosing a suitable cell line for bone regeneration, a number of factors should be considered. These include viability for mass production, low antigenicity of ECM components, and elimination of infectious agents. An immortalized cell line that can be expanded indefinitely to produce bone generating cells would realize these goals.

Potential sources for a suitable cell line include immortal cells from tumors such as osteogenic sarcoma or ossifying fibroma, embryonic stem cells, or mesenchymal stem cells. Immortalized cells from tumors would be easily obtainable and relatively easy to culture indefinitely. However, a concern is that despite decellularization, the ECM could harbor proteins or other products that promote tumorigenesis. Bone sourced from embryonic stem cells presents a number of ethical concerns. Native sources of bone precursor stem cells, particularly mesenchymal stem cells (MSCs) from bone marrow or dental pulp, have been shown to be good candidates for bone generation and may be suitable as an industrialized supply line for commercialization.




A newer option is induced pluripotent stem cells (iPSCs). iPSCs share many characteristics with embryonic stem cells, but without the associated ethical concerns, and have been rapidly adopted by the research community. Recent studies[4] show some limitations with iPSCs in their ability to differentiate into targeted tissue, and further research is needed to establish them as an optimal source for a cell line. Any differentiated cell line from iPSCs must be carefully screened to ensure elimination of problematic cell populations.

Finally, genetic alteration using CRISPR biotechnology can enable researchers to tailor desirable features into a cell line; for example, enhancing the production of bone morphogenic protein (BMP) in the final product. However, these modifications are still in experimental stages and will require animal testing to ensure safety. In addition, synthetic bone grafts enhanced with added BMP have been shown to increase cancer incidence in some patient populations.

## Scaffold

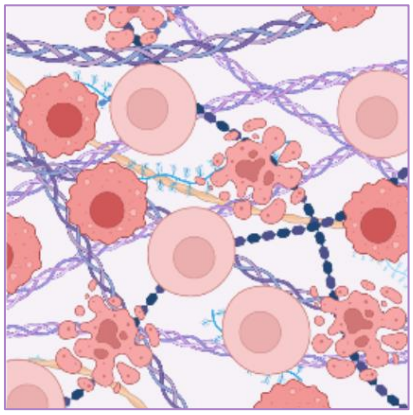
When culturing cells, certain cell types like fibroblasts, keratinocytes, and osteo-progenitors require a physical surface to attach to in order to survive. For bone production from precursor cells, a physical scaffold is required for cell attachment. Much research has been done on the performance of various scaffold innovations. An optimal scaffold must be cost-effective and easy to seed with stem cells. It must also promote osteogenesis, and should be moldable into various shapes for different surgical applications.

One popular and well-studied scaffold material is collagen hydrogels. MSCs are seeded onto collagen hydrogels and a culture media is added to feed the cells, along with agents that promote bone differentiation and production. A problem often encountered herein is optimizing scaffold diffusion for the delivery of nutrients and removal of waste products for cell survival. The thicker the scaffold, the more difficult diffusion becomes. Pre-vascularized engineering of hydrogels is being investigated as a potential solution, as is 3D tissue printing of vascularized tissues. These methods could allow culture of thicker and more densely cellularized scaffolds, yielding greater end product.

A number of other matrices have been developed and studied, including ceramics, polymers, and metals. A mixture of hydroxyapatite (HA) and calcium carbonate can be tailored to have a degradation rate similar to the rate of new bone formation. This allows the scaffold to be replaced with bone in vitro before final processing, a key feature for scaffold optimization.

## Culture and Mass Production

Scaffolds are commonly seeded with cells of various densities prior to culture. The seeded scaffold is then loaded into a bioreactor that contains a culture media. There are many bioreactors designed for bulk culture, with an overall goal to maximize production of a bone ECM that closely mimics natural bone. Agitation and flow of media are essential, as nutrient flows in static culture vessels have shown penetration of only 1mm or less. Rotating wall vessel (RWV) bioreactors or perfusion reactors perform the best. However, both have relatively low capacity for mass production, and novel methods are needed with solutions like slow-release glucose.



A decellularized ECM

## Decellularization

The aim of decellularization is to remove the cell contents while preserving the ECM and osteogenic compounds. The cell contents are removed to prevent immune reactions or pyogenic reactions, and, in the case of bone sourced from tissue banks, to remove infectious agents. The ECM is the component that is osteoconductive and osteoinductive, and is spared from the immune response.

Decellularized bone grafts are easier to store and transport, which reduces costs and extends shelf life. Minimal steps like freeze-thawing, sonication, and/or a carbon dioxide supercritical fluid process are

needed to remove the cellular component. These methods preserve the proteins and other organic components in the ECM. No industry standards yet exist for stem cell-derived bone, and research to optimize these protocols for industrialization and commercialization is ongoing.

## Sterilization

Compared to bone sourced from tissue banks, stem cell-derived bone grown in a GMP-certified facility under aseptic conditions has no risk of infection, as the cell line is prescreened for harmful agents. Bone sourced from tissue banks commonly undergoes treatments like gamma radiation or autoclaving, which are harsh and destroy many of the desired organic components for inducing bone regeneration. Ethylene oxide or CO2 supercritical fluid may be better-suited for lab-grown bone.

## Regulation

Allogenic bone grafts are classified as an FDA 510(k) Class II medical device. To ensure their safety and biocompatibility, they must be tested in accordance with ISO guidelines. If the raw materials, manufacturing, sterilization, and packaging of a bone graft product are all identical to those of another cleared product currently on the market, 'substantial equivalence' has been met and no additional biocompatibility testing is required. However, it remains to be seen how allogenic products generated in vitro using stem cells will be treated by the FDA, as few have reached this point in their development.

# Conclusion

A stem cell-derived bone graft must overcome considerable hurdles before it can be made available to the public. Among these are selection of an optimal cell line and scaffold to facilitate nutrient delivery and bone regeneration; a tissue culture method conducive to mass production; and thorough decellularization and sterilization efforts to prevent immune reaction without destroying the ECM. Such a challenge requires the creation of a holistic manufacturing process and establishment of standardized production protocols. The overarching goal, of harnessing mother nature's innate capabilities to better human health, is within reach.

# References


[1] Bone Grafts and Substitutes in Dentistry: A Review of Current Trends and Developments

[2] Manufacturing artificial bone allografts: a perspective

[3] Multicenter study of patients' preferences and concerns regarding the origin of bone grafts utilized in dentistry.

[4] Age Is Relative-Impact of Donor Age on Induced Pluripotent Stem Cell-Derived Cell Functionality