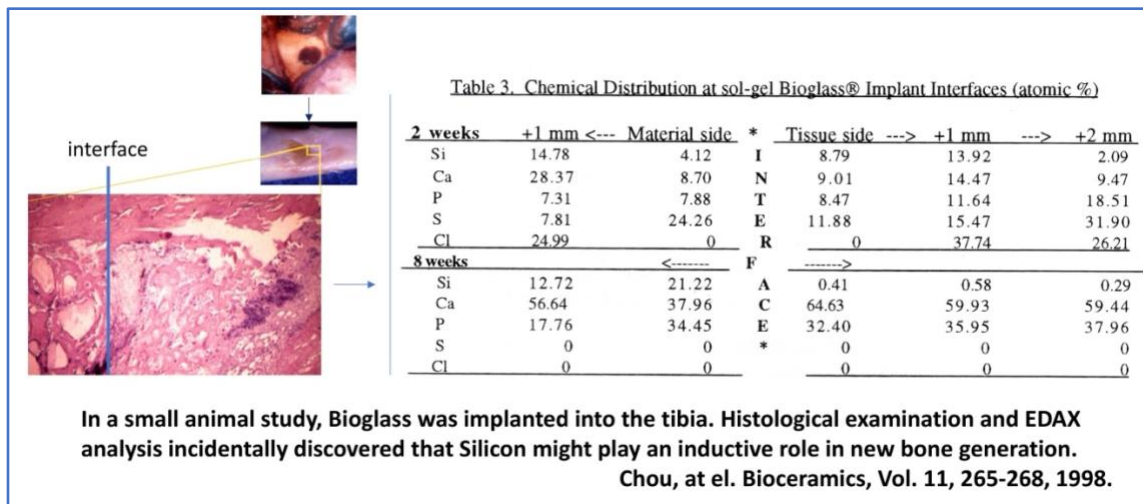


# The First 3-D Printing Device and First Biomimetically Engineered Scaffold benefited Over Thousands of Patients

## 1) Invention of Osteogenic Materials, Biomimetic Scaffold, and the First 3-D Printing Device for Tissue Engineering

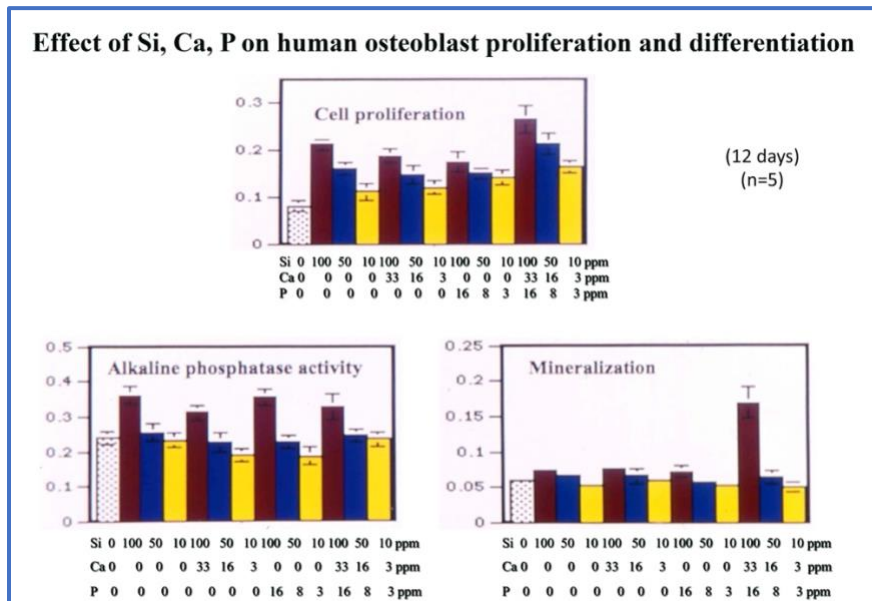
### a) The osteogenic role of Silicon first discovered by Dr. Chou in 1995

In 1995, in a histological examination of animal tissue with implanted materials under the microscope, Dr. Chou discovered incidentally the dynamic profile at the tissue/implant interface. He noticed the active new bone formation in the area about 2 millimeters distant from the interface line. Further SEM and EDAX studies confirmed that Silicon released from the implanted material to the distant zone of tissue strongly correlated to active bone formation.



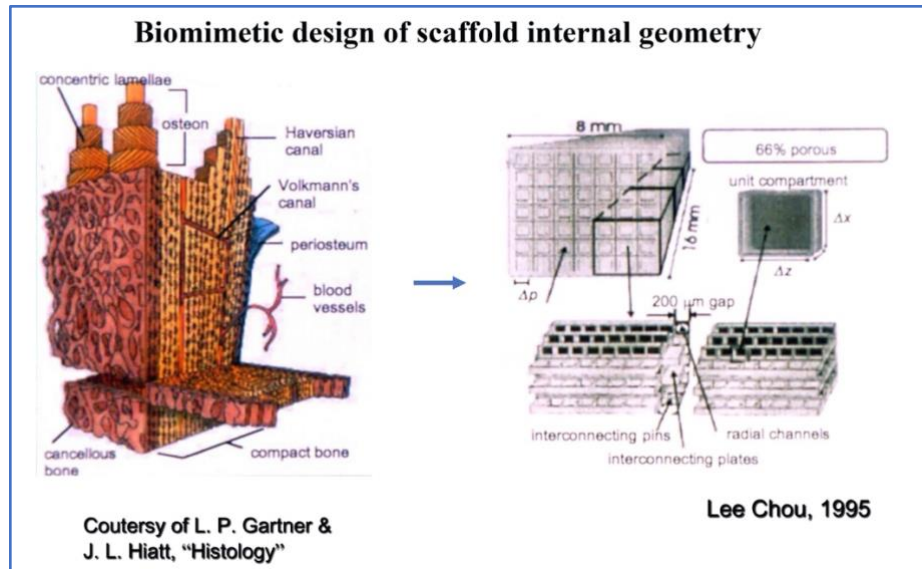
In order to further confirm this exiting discovery, in 1995, Dr. Chou conducted a series of experiments using normal human osteoprogenitor cells and supplemental Si, Ca, and P in different concentrations and combinations. The results showed the osteogenic effect of silicon and synergistic effect of calcium on stimulating bone regeneration via upregulation of osteoprogenitor cell proliferation, alkaline phosphatase activity and mineralization as showing in below figure. This breakthrough finding was the first time to set the foundation of Si and Ca as stimulatory elements in biomaterials for medical applications. This is also

the foundation of many of Dr. Chou's further inventions in bioactive materials and scaffolds for skin wound healing and bone tissue engineering.



### b) The first biomimetic scaffold designed by Dr. Chou for bone tissue engineering


For many years, the scaffolds for tissue engineering were designed in a homogenized pattern with certain percentages of pores, which inhibited the cell distribution and blood vessel ingrowth evenly in the entire scaffold, resulting in failures of bone regeneration. Dr. Chou made the first biomimetic design of bone tissue engineering scaffold in 1995 with the specific boxes for bone tissue nucleation, specific channels for cell seeding and specific channels for blood vessel ingrowth. This unique design of scaffold internal geometry would be able to facilitate the cell seeding, cell distribution, bone growth and blood vessel ingrowth, allowing the bone regeneration for large defect of the bone.



**c) The first 3-D printing device developed by Dr. Chou for tissue engineering**

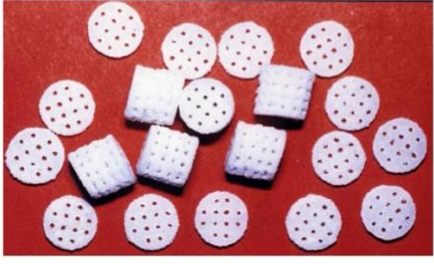
After discovery of osteogenic materials and biomimetic design of scaffold internal geometry, in order to fabricate the sophisticated scaffold at the scale of micrometers, Dr. Chou developed the first 3-D printing device for tissue engineering in 1995. At that time, any commercial 3-D printing device was not available for tissue engineering. With this hand-made 3-D printing device as showed below, the osteogenic scaffolds with biomimetically designed internal geometry were successfully fabricated.

### World first 3D printing machine for tissue engineering in 1995

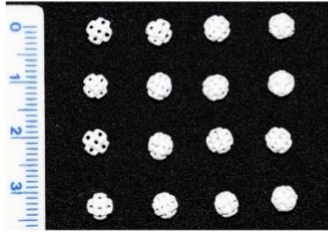


**3-D device**

L. Chou



**Scaffolds printed**



# Research

at Boston University

2005







of healing will be done in one week. Although the research thus far has been conducted *in vivo* and several years of testing will lie ahead, he hopes that the nanostructure surfaces will one day be used for human implants.

To create the nanostructures, Popat coats one side of an aluminum sheet with a polymer similar to nail polish. He places the sheet in a reaction chamber along with a similar-sized platinum sheet and immerses them in acid, then runs an electric current through them. A layer of aluminum oxide grows on the uncoated side, creating a grid of tiny nanopores. By regulating the electrical current, Popat can control the pore size and distribution, allowing him to create nanostructure at a scale comparable to that in natural bone. After about eight hours, the oxidized aluminum sheet is removed and put into chromic acid, which etches the oxide to create regular porous nanostructures. A final copper chloride bath can strip away any remaining extraneous aluminum, leaving behind a thin nanoporous membrane. This membrane can be used to coat existing orthopedic implants, providing a more welcoming surface for the growth of natural bone cells.

Scientists use similar technology to create porous membranes for filtration devices, but according to Popat this is the first time the technology has been used for orthopedic implants. He suggests that another practical application for the nanopore membranes could be drug delivery systems in which the pores would be employed to gradually release drugs into the body.

**REGROWING BONE**

Like Keral Popat, Lee Chou thinks that implants should provide a comfortable home for bone cells. However, he takes this idea one step further—biodegradable scaffold implants that grow bones where no bones have grown before.

Scientists have traditionally believed that inert materials made the best implants. Since materials such as titanium barely react or change within the body, the body doesn't react negatively to them. Chou, on the other hand, who specializes in biomaterials and oral medicine at the Goldman School of Dental Medicine, believes an implant made of bioactive, as opposed to inert, materials will coax the body into reacting positively to it.

On a molecular level, no material is completely inert in the body, says Chou. The unique chemical makeup and shape of the surface always causes biological changes, even changing the way genes are expressed. Orthopedists usually use special proteins to induce cell growth on conventional implants. However, says Chou, those genetically engineered proteins change when they come in contact with the implant—the implant and proteins were never designed to work in tandem.

Chou's approach uses the chemistry and shape of the implanted scaffold to control genes and induce bone growth. He spent several years screening different materials in the research lab before he hit on the right combination of elements.

In addition to the cell-growth-stimulating chemistry of the scaffold, the structure, which looks somewhat like a hard piece of Styrofoam, provides an attractive home for bone cells. The hollow, porous design allows bone cells to grow inside, around, and on the scaffold; and blood tends to grow through it, in contrast to conventional implants that only allow cells to grow on the surface. Chou also designed the scaffold to dissolve after the bone grows in, leaving only natural bone behind.



**Lee Chou**, who teaches in the departments of Restorative Sciences and Biomaterials at the Goldman School of Dental Medicine, has developed innovative three-dimensional biodegradable bone implants that encourage the growth of bone cells. Chou's implants have provided a structure on which bone tissue has grown in places where no bone previously existed.



**Keral Popat** has applied a technology used to fabricate porous filtration membranes to create bone-friendly metal implants. The surfaces of the implants are covered with nano-sized pores (magnified 300,000 times) that mimic the surface of real bone and encourage rapid healing.

Chou implants the scaffold into a body and adds osteogenic cells taken from a chip of the patient's own bone. He's had astounding results. One of his patients was born without upper jawbones. Within three months, the scaffold had created useable jawbones, allowing the man to eat normally and flash a smile for the first time in his 26 years. Astonishingly, although the patient was born with a genetic disease that makes his bones naturally brittle and Chou used cells from his genetically defective bones to seed the scaffold, the structure coaxed them to grow normal, healthy bones—bones even stronger than his natural bones.

At the moment, Chou and his collaborator David Cottrell, chairman of the Department of Oral and Maxillofacial Surgery, are focusing on additional clinical studies for using bone tissue engineering to treat patients with severe bone defects. Although Chou concentrates his research on dental applications, he envisions that his bioactive scaffold will one day be used to grow bones throughout the body.

A man of many interests and talents, Chou also directs a project focusing on the mechanism by which the body takes in the AIDS virus. He also has been recognized for excellence in teaching—in 2002 he received BU's highest teaching award, the Merzall Cup and Prize.

—Eliana Hayashi

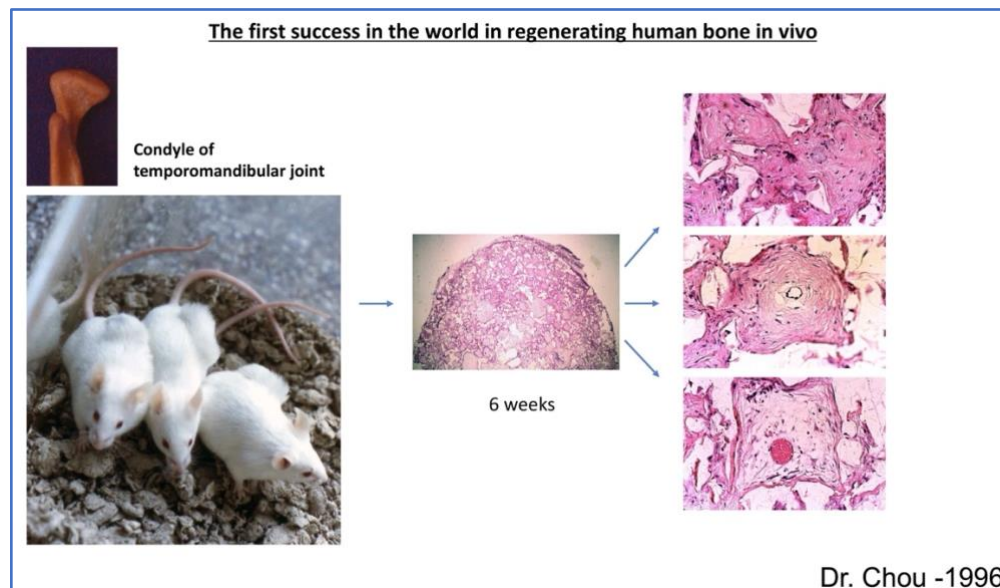
Research at Boston University 2005 23

## 2) In Vivo Studies of Bone Tissue Engineering

### a) The first engineered human bone tissue

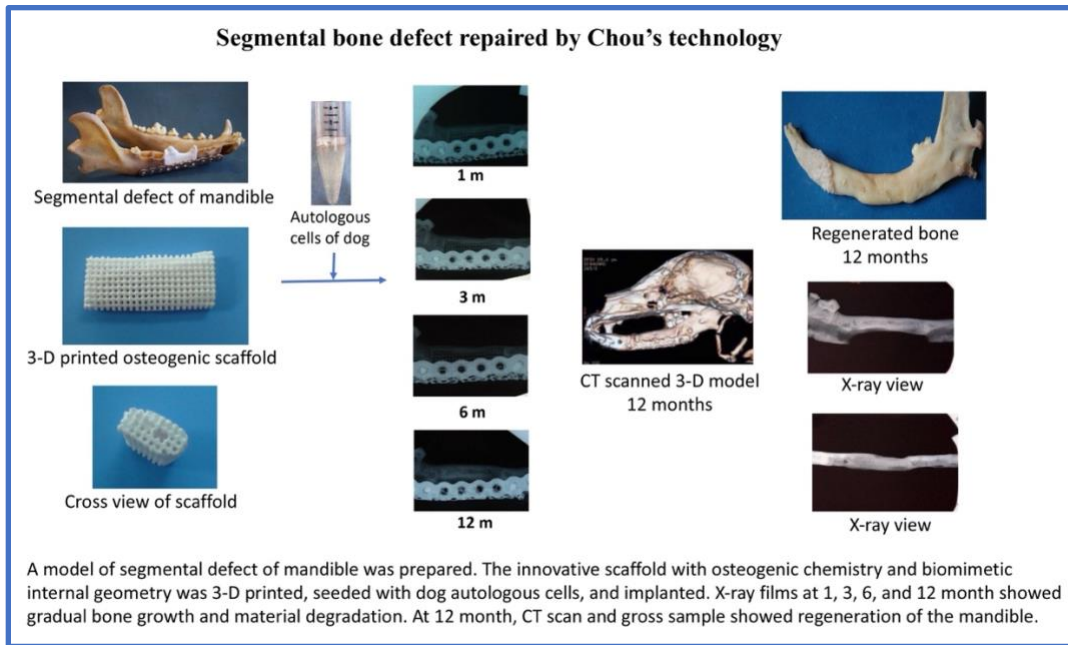


In 1996, Dr. Chou successfully engineered the human bone using the scaffold with his innovative osteogenic materials and biomimetic design of internal geometry. A large scaffold seeded with human osteoprogenitor cells derived from a healthy patient donor was implanted into SCID mice under Institution IACUC approval. As showing below figures, at 6 weeks of implantation, bone tissue regrowth throughout the entire scaffold of 1 cm in size. High magnification showed high quality of woven bone (primary bone) formation, Haversian structure, blood vessel ingrowth, and degradation of scaffold materials. **This was the first success in the world in regenerating human bone in vivo.**



### **b) The first success in repairing a segmental bone defect**

One of the major challenges in bone tissue engineering is to repair the segmental defect of load-bearing bones. In 2002, Dr. Chou first made a breakthrough work in reconstructing the mandible in a dog with segmental defect. Combined with his four innovative technologies: osteogenic active biomaterial, biomimetic design of scaffold internal geometry, 3-D printing, and autologous osteoprogenitor cell technology, Dr. Chou successfully regenerated the new mandible in a dog with over critical sized segmental defect.



### 3) Translating Inventions into a Series of Products Named **OSTEOBONE**

Dr. Chou has translated his discoveries and inventions into a series of products named **OSTEOBONE** for medical application.



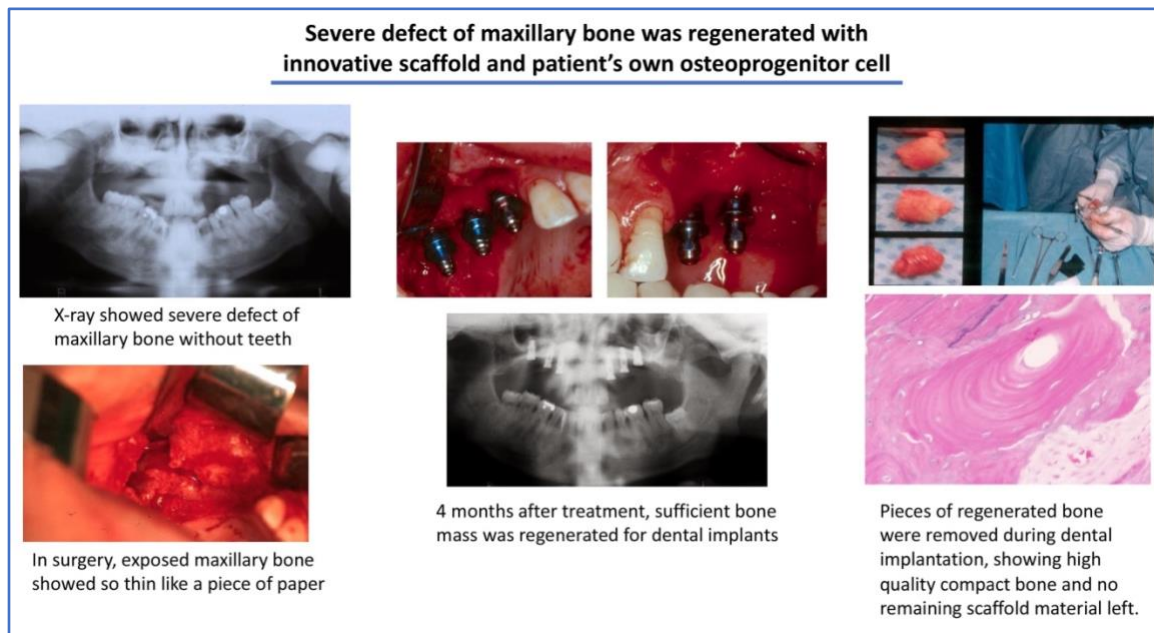
**OSTEOBONE™** is a series of innovative products developed by Dr. Chou, covered by 19 patents. **OSTEOBONE™** has successfully treated over fourteen thousands of patients with various kinds of bone defects including segmental bone defects, genetically inherited bone defects, server bone fractures, donor site bone repair, and bone defects after bone tumor removal for regenerating bone by virtually zero short- or long-term failure rates after over 20 years of post-operation follow-up.

#### 4) Selected Patients Treated with **OSTEOBONE** Bone Scaffold Products

##### a) The first patient with an over 25-year long-term follow-up after treatment with Chou's innovative technique in 1998

This was the first case successfully treated by Dr. Chou's innovative technologies of osteogenic materials, biomimetic design of scaffold internal geometry, 3-D printing device and patient's autologous osteoprogenitor cells under the IRB approval in 1998, as otherwise no other effective choice of treatment was not available.

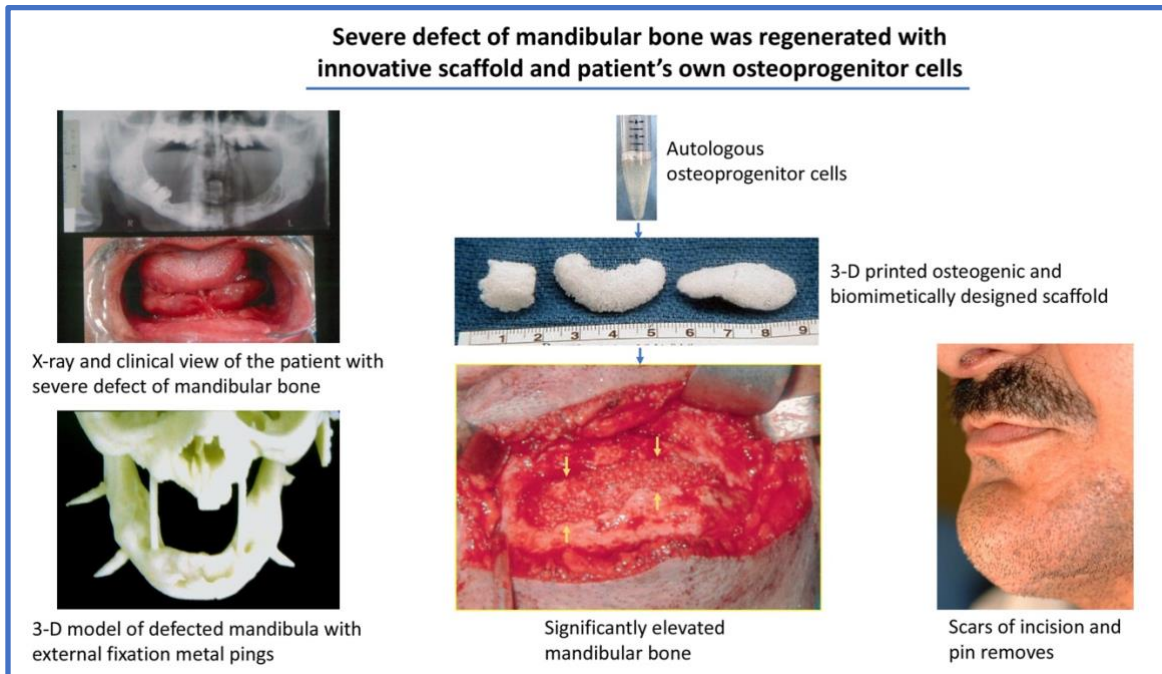
This patient of 26 years of age suffered with benign dominant osteopetrosis with significantly atrophy of his maxilla without posterior teeth. After his maxillary bone was regenerated, dental implants were placed with full function. Now, this patient has been followed up for over 25 years. All implants placed remain functional, indicating the long-term success. *(Patient identity is intentionally concealed)*



##### b) The second patient with an over 25-year long-term follow-up after treatment by Chou's innovative technique in 1999

This was the second case successfully treated by Dr. Chou's inventive technologies of osteogenic materials, biomimetic design of scaffold internal geometry, 3-D printing device and patient's autologous osteoprogenitor cells under the IRB approval in 1999.

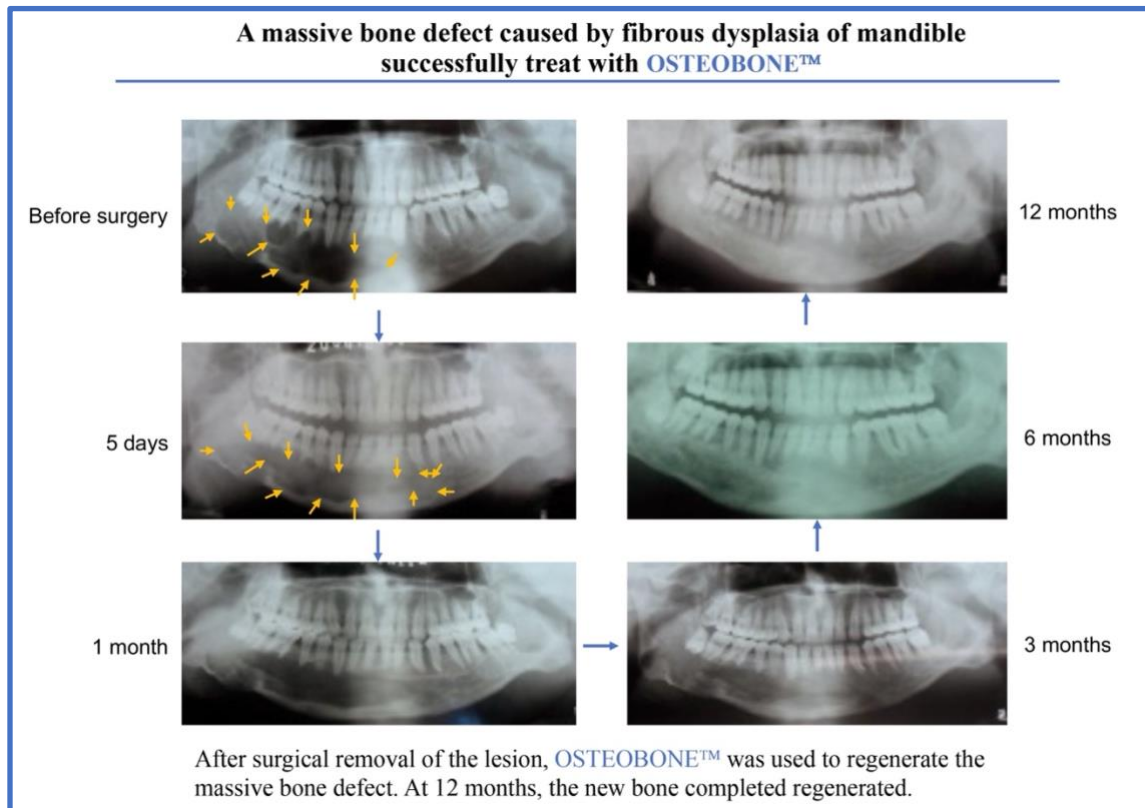
This patient of 45 years of age suffered with benign dominant osteopetrosis with significantly atrophy of his mandibular bone without any functional tooth. He also suffered with frequent fractures of his mandibula, so needed the external fixations with four external metal pins. After his mandibular bone was regenerated, fracture never occurred without external fixation. Now, this patient has been followed up for over 25 years. His masticatory function remains completely normal without any external fixation, indicating the long-term success. *(Patient identity is intentionally concealed)*



### c) Large mandible defect successfully treated with **OSTEOBONE**

This patient suffered with massive lesion of fibrous dysplasia of mandible. Routinely, a mandibulectomy would be performed in surgical removal of the lesion because of the size of defect. With the treatment of **OSTEOBONE**, the massive defect was completely filled with newly generated bone in 12 months. His mandible was saved to secure the quality of his life. *(Patient identity is intentionally concealed)*



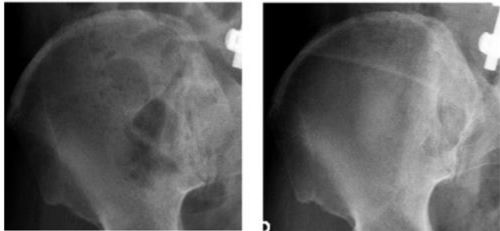


#### **d) Bone donor site repaired with OSTEObONE**

This patient was one of the large-scale clinical trials for treatment of the bone donor site defects with OSTEObONE in comparison to the conventional treatment with hydroxyapatite (HA). The levels of material degradation and new bone formation are the measures of success. According to the FDA regulation, the trial was conducted by the independent multi-centered institutions. The results showed that OSTEObONE was significantly superior than HA in both material degradation and new bone formation ( $P < 0.0001$ ). This patient presented below is an example of the trial group. *(Patient identity is intentionally concealed)*

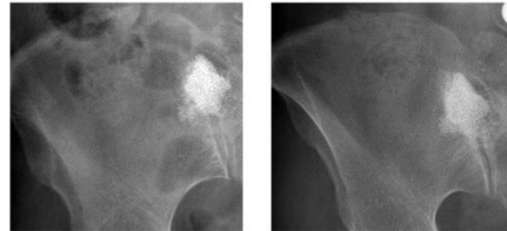
## Comparing **OSTEOBONE™** with HA in treating iliac ridge donor site defects

Case A - treated with **OSTEOBONE™**



1 week after surgery    6 months after surgery

Case B - treated with **HA**



1 week after surgery    6 months after surgery

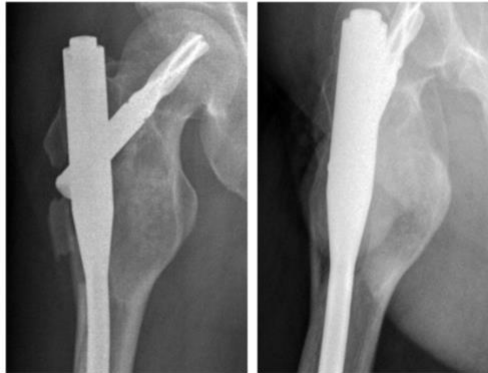
The X-ray films indicated that, at 6 month, Case A with **OSTEOBONE™** showed a complete regrowth of the trabecular bone while Case B with **HA** showed no change in implanted materials and no new bone formed

### e) **Bone defect after benign bone tumors treated with **OSTEOBONE****

This patient was one of the large-scale clinical trials for treatment of defects after benign bone tumor removals with **OSTEOBONE** in comparison to the conventional treatment with hydroxyapatite (HA). The levels of material degradation and new bone formation are the measures of success. According to the FDA regulation, the trial was conducted by the independent multi-centered institutions. The results showed that **OSTEOBONE** was significantly superior than HA in both material degradation and new bone formation ( $P < 0.0001$ ). The patient presented below is an example of this trial group. *(Patient identity is intentionally concealed)*

### Comparing **OSTEOBONE™** with HA in treating the defects after benign bone tumor removal

Case A - treated with **OSTEOBONE™**



1 week after surgery

6 months after surgery

Case B - treated with **HA**



1 week after surgery

6 months after surgery

The X-ray films indicated that, at 6 month, Case A with **OSTEOBONE™** showed a complete regrowth of the trabecular bone while Case B with **HA** showed no change in implanted materials and no new bone formed.

#### f) Bone fracture treated with **OSTEOBONE**

This patient was one of the large-scale clinical trials for treatment of defects bone fractures with **OSTEOBONE** in comparison to the conventional treatment with hydroxyapatite (HA). The levels of material degradation and new bone formation are the measures of success. According to the FDA regulation, the trial was conducted by the independent multi-centered institutions. The results showed that **OSTEOBONE** was significantly superior than HA in both material degradation and new bone formation ( $P < 0.0001$ ). The patient presented below is an example of this trial group. *(Patient identity is intentionally concealed)*

## Comparing **OSTEOBONE™** with HA in treating bone fractures

Case A - treated with novel material



1 week after surgery    6 months after surgery

Case B - treated with HA



1 week after surgery    6 months after surgery

The X-ray films indicated that, at 6 month, Case A with **OSTEOBONE™** showed a complete regrowth of the trabecular bone while Case B with **HA** showed no change in implanted materials and no new bone formed.

### 5) Patents Covering the Invention and Production of **OSTEOBONE**

Human Bone Tissue Engineering Scaffold and Its Preparation and Application. China. Publication Number: CN1294885C, Patent Number: ZL 01113076.8, 2007.

Bone Tissue Repair Material Delivery Device for Minimally Invasive Surgery. Publication Number: CN2840998Y, Patent Number: ZL200520076281.5, 2006.

Human Bone Tissue Engineering Scaffold and Its Preparation and Application. United Kingdom, France, Germany, Belgium, Spain, Sweden, and Italy. Publication number: 02742644.4-2107, Patent Number: 1426066. 2010.

Human Bone Tissue Engineering Scaffold and Its Preparation and Application. Japan. Publication Number: 2003-501511, Patent Number: 4391815, 2009.

Human Bone Tissue Engineering Scaffold and Its Preparation and Application. Russia. Publication Number: 200313782.3, Patent Number: 2308974, 2007.

Ingredient Tanks for Production of Bone Repairing Materials. China. Publication Number: CN219744676U, Patent Number: ZL202320694223.7, 2023.

Crusher for Production of Bone Repairing Materials. China. Publication Number: CN219744896U, Patent Number: ZL202320382589.0, 2023.



Scaffold for Repairing Large Mandible Defect. China. Publication Number: CN1143668518B, Patent Number: ZL202111679163.3, 2022.

A Cutting Device for Bone Repairing Materials. China. Publication Number: CN214724555U, Patent Number: ZL202023267401.3, 2021.

Tissue Engineering Scaffold for Repairing Large Skull Defects. China. Publication Number: CN215019736U, Patent Number: ZL202023192408.3, 2021.

A Reinforcement Device for Tissue Engineering Scaffold for Repairing Large Skull Defects. China. Publication Number: CN215019737U, Patent Number: ZL202023192409.8, 2021.

Bone Repairing Scaffold Material Mixing Device. China. Publication Number: CN210906376U, Patent Number: ZL201921321524.5, 2021.

A Spray Humidifying Device for Bone Repairing Materials. China. CN210906606U, Patent Number: ZL201921321538.7, 2020.

Cuttable Bone Scaffold. China. Publication Number: CN207445077U, Patent Number: ZL201720363539.2, 2018.

Operation Cabinet for Bone Repairing Scaffolds. China. Publication Number: CN206840152U, Patent Number: ZL201720363587.1, 2018.

Humidifying Spray Device for Bone Tissue Defect Filling Materials. China. Publication Number: CN206838335U, Patent Number: ZL201720346785.7, 2018.

Scaffold for Bone Repairing. China. Publication Number: CN207462190U, Patent Number: ZL201720346757.5, 2018.

Surgical Instruments for Bone Defect Volume Measurement and Repairing. China. Publication Number: CN201768022U, Patent Number: ZL201020286208.1, 2011.

Improved Bone Tissue Repairing Material Delivery Device for Minimally Invasive Surgery. China. Publication Number: CN201019818Y, Patent Number: ZL200720033333.X, 2008.

## **6) Articles Published by the Independent Clinicians Who Used [OSTEOBONE](#) in Their Clinics with Successful Outcomes**

**OSTEOBONE versus bone autograft for calcaneal fractures accompanied with bone defect**  
G. Yi, J. Yang, L. Zhang, S. Fu, X. Guo, Y. Liu, B. Qin, G. Wang. Orthopedic Surgery of China. Vol. 8, 706-711, 2019.

Abstract: To compare the clinical outcomes of **OSTEOBONE** versus bone autograft, 72 patients of calcaneal fractures accompanied with bone defect from January 2014 to January 2017 in our hospital were randomly divided into two groups, a study group of 37 patients received artificial **OSTEOBONE** (AB), control group of 35 patients treated with bone autograft (BA). Results: The AB group proved significantly superior to the BA group regarding to the operation blood loss, operation time and postoperative drainage ( $P < 0.05$ ). The follow up period lasted for more than 1 year. No significant difference was proved in fracture healing time between the two groups ( $P > 0.05$ ). The AOFAS scores significantly increased as time went on in both groups ( $P < 0.05$ ). Conclusion: The **OSTEOBONE** as an alternative to bone autograft does achieve satisfactory clinical outcomes with few complications and is suitable to calcaneal fractures accompanied with bone defect.

<https://cstj.cqvip.com/Qikan/Article/Detail?id=90748883504849574856484857&from=Qikan Article Detail>

### **Gray Scale Ratio Evaluation of the Effectiveness of **OSTEOBONE** Repair Materials in Repairing Mandibular Bone Defects**

X. Luo, Y. Wang. Chinese Journal of Aesthetic Medicine. Vol.3, 114-117, 2018.

Abstract: To evaluate the osteogenic effect of **OSTEOBONE** repair material through the changes in imaging grayscale ratio after it is implanted into the mandibular defect, 20 patients with bone defects in the posterior mandibular area due to odontogenic cysts were selected and implanted with **OSTEOBONE** repair materials. CBCT was taken before surgery and at 1, 3, and 6 months after surgery. The grayscale ratio of the normal and coronal planes and the healthy side was measured. Conclusion: **OSTEOBONE** repair material has a significant osteogenic effect in repairing bone defects in the posterior mandibular area.

<http://www.cqvip.com/qk/90129a/201803/7000560621.html>

### **Clinical Efficacy of **OSTEOBONE** Repair Materials in GBR at the Same Time as Extraction of Impacted Mandibular Third Molars**

W. Chen, Z. Chen, W. Zhang, K. Chen. Journal of Clinical Stomatology. Vol. 7, 421-423, 2019.

Abstract: To observe and analyze the clinical efficacy of **OSTEOBONE** repair materials in guided bone re-generation (GBR) during the extraction of impacted mandibular third molars at the same time, 40 patients with impacted mandibular third molars were treated in randomly divided GBR group and control group (20 cases each). The GBR group underwent guided bone tissue regeneration using **OSTEOBONE** repair materials after tooth extraction, while the control group underwent routine treatment of tooth extraction wounds with only tight suturing. Records were recorded 6 months after surgery. The bone density of the tooth extraction area, the change

in the distal alveolar ridge height of the second molar and the pain were measured in the two groups. **Results:** Bone density (Hu) of the **OSTEOBONE** group ( $137.35\pm 8.82$ ) was higher than that of the control group ( $109.10\pm 11.40$ ); The change in the height of the distal alveolar ridge of the second molar (H) in the **OSTEOBONE** group ( $4.56\pm 1.43$ ) mm was higher than that in the control group ( $0.93\pm 0.83$ ) mm; the visual analog scale value was significantly smaller than that of the control group ( $2.00\pm 1.15$ ) ( $P<0.01$ ). **Conclusion:** **OSTEOBONE** repair material can be used at the same time after the extraction of mandibular impacted third molars. The therapeutic effect is remarkable with **OSTEOBONE**.

[https://www.nstl.gov.cn/paper\\_detail.html?id=5c6498d3594138f31b6af493e5143f3d](https://www.nstl.gov.cn/paper_detail.html?id=5c6498d3594138f31b6af493e5143f3d)

[https://xueshu.baidu.com/usercenter/paper/show?paperid=1p4f0vs0aj300a70ux4w02e0c2032862&site=xueshu\\_se](https://xueshu.baidu.com/usercenter/paper/show?paperid=1p4f0vs0aj300a70ux4w02e0c2032862&site=xueshu_se)

### **Synchrotron radiation imaging to compare the effectiveness of two different bone repair materials in repairing femoral defects in rabbits**

L. Sun, Y. Liu, Z. Wang. Chinese Journal of Tissue Engineering Research. Vol. 27(21), 3343-3348, 2023.

**Abstract:** To compare the effectiveness of Bio-Oss bone powder and **OSTEOBONE** bone repair materials in repairing bone defects using synchrotron radiation micro-tomography imaging and histopathologic examination, 32 white rabbits were randomly divided into a control group ( $n=12$ ) and an experimental group ( $n=20$ ). The control group was filled with physiological saline, and the experimental group was filled with **OSTEOBONE**. Eight weeks after the operation, the repair effects of the two repair materials were evaluated through gross observation, imaging, and histopathological examination. **Results and conclusion:** The CT value of **OSTEOBONE** side at 8 weeks was significantly higher than that of the Bio-Oss bone powder side ( $P<0.05$ ). The results show that **OSTEOBONE** repair material can promote the formation of new bone and damage repair.

[https://www.sohu.com/a/610418491\\_120582835](https://www.sohu.com/a/610418491_120582835)

[https://xueshu.baidu.com/usercenter/paper/show?paperid=146a00e0n47n0ef0vb050rp0r1045594&site=xueshu\\_se](https://xueshu.baidu.com/usercenter/paper/show?paperid=146a00e0n47n0ef0vb050rp0r1045594&site=xueshu_se)

### **Comparison of Clinical Efficacy of the Double-Incision Locking Plate Combined with **OSTEOBONE** Implantation and Autogenous Iliac Bone Graft in the Treatment of Complex Tibial Plateau Fractures**

G. Yi, L. Zhang, S. Fu, X. Guo, Y. Liu, B. Bo, Y. Luo, G. Wang. Chinese Journal of Tissue Engineering Research. Vol. 23(16), 2486-2492, 2019.

**Abstract:** To compare the clinical efficacy of double-incision locking plate combined with **OSTEOBONE** implantation and autologous iliac bone graft in the treatment of complicated tibial plateau fractures, clinical data of 71 patients with complex tibial plateau fractures who underwent open reduction and internal fixation with double-incision locking plate and bone graft were analyzed retrospectively. Among them, 35 cases were treated with double-incision locking plate combined with **OSTEOBONE**, and 36 cases (control group) were treated with double-incision locking plate combined with autologous iliac bone graft.

**RESULTS AND CONCLUSION:** Follow-up results showed that the operation time, intraoperative blood loss, postoperative drainage volume, fracture healing time, pain and symptom points in Knee Injury and Osteoarthritis Outcome score, collapse score and total score

in Rasmussen imaging, and incidence of complications in **OSTEOBONE** group were significantly better than those in the control group ( $P < 0.05$ ). These results imply that compared with the autologous iliac bone graft, the treatment of complex tibial plateau fractures with the double-incision locking plate internal fixation combined with **OSTEOBONE** can shorten the operation time, reduce bleeding and complications, form a stronger support, and accelerate fracture healing.

<https://www.cjter.com/CN/10.3969/j.issn.2095-4344.1204>