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 millimets 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 corelated to active bone formation.

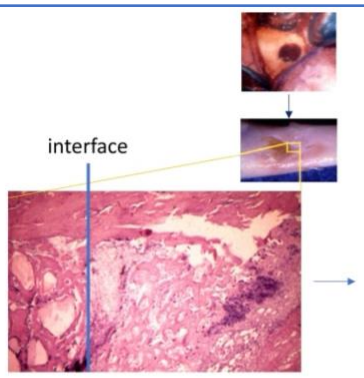


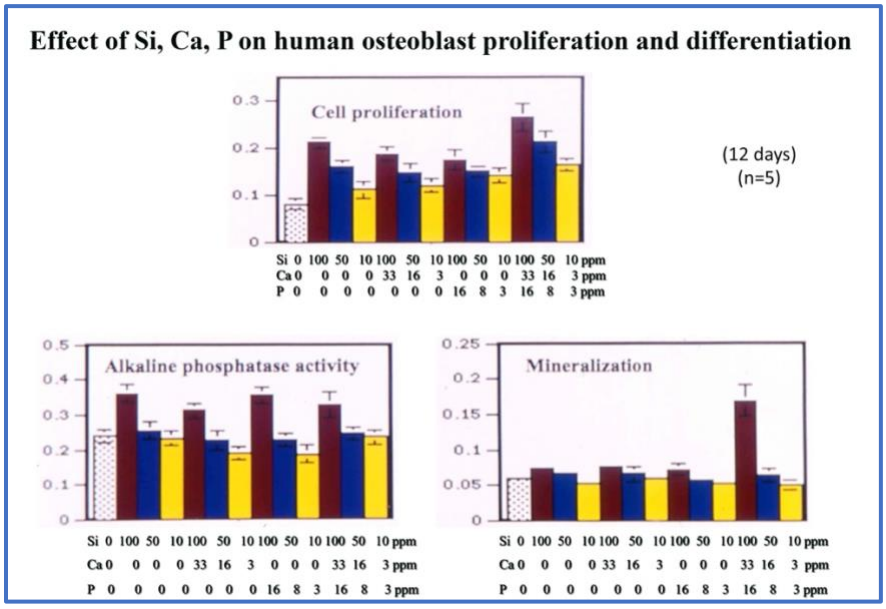
Table 3. Chemical Distribution at sol-gel Bioglass® Implant Interfaces (atomic %)

2 weeks	+1 mm <---	Material side	*	Tissue side --->	+1 mm --->	+2 mm
Si	14.78	4.12	I	8.79	13.92	2.09
Ca	28.37	8.70	N	9.01	14.47	9.47
P	7.31	7.88	T	8.47	11.64	18.51
S	7.81	24.26	E	11.88	15.47	31.90
Cl	24.99	0	R	0	37.74	26.21
8 weeks		<-------	F	------->		
Si	12.72	21.22	A	0.41	0.58	0.29
Ca	56.64	37.96	C	64.63	59.93	59.44
P	17.76	34.45	E	32.40	35.95	37.96
S	0	0	*	0	0	0
Cl	0	0		0	0	0

In a small animal study, Bioglass was implanted into the tibia. Histological examination and EDAX analysis incidentally discovered that Silicon might play an inductive role in new bone generation.
Chou, at el. Bioceramics, Vol. 11, 265-268, 1998.

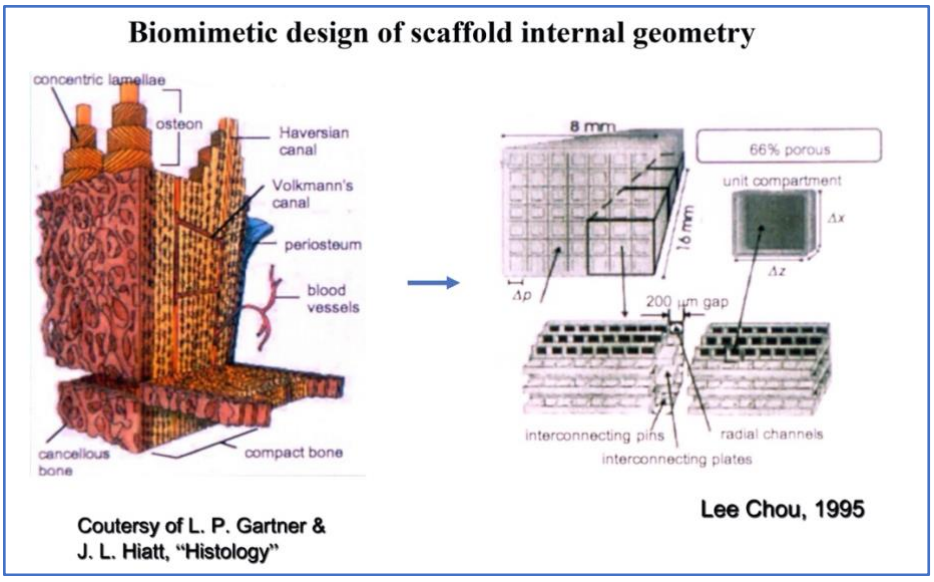
In order to further confirm this exiting discovery, in 1995, Dr. Chou conducted a series of experiments using normal human osteoprogenitor cells and supplemental Si, Ca, and P in different concentrations and combinations. The results showed the osteogenic effect of silicon and synergistic effect of calcium 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.



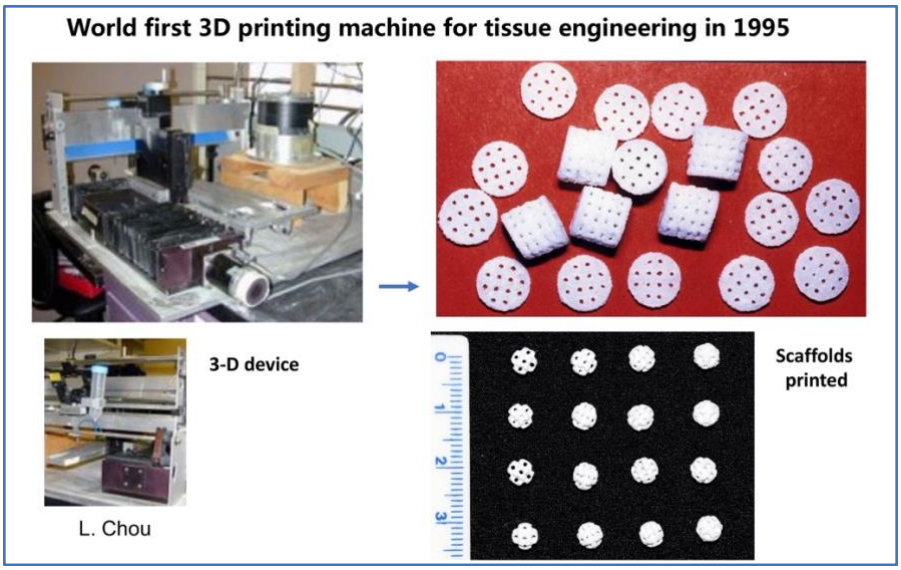
b) The first biomimetic scaffold designed by Dr. Chou for bone tissue engineering

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

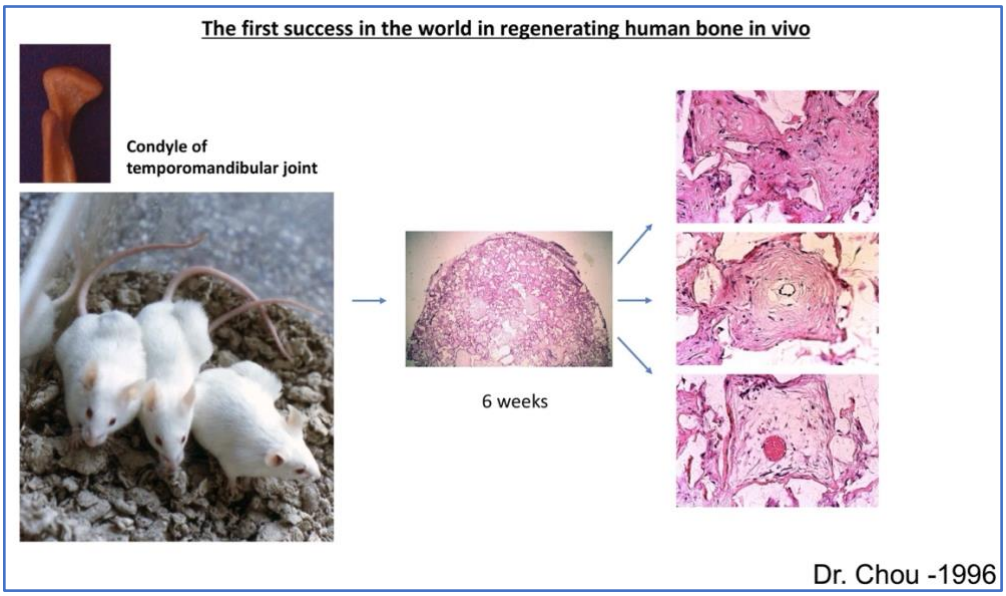
After discovery of osteogenic materials and biomimetic design of scaffold internal geometry, in order to fabricate the sophisticated scaffold at the scale of micrometers, Dr. Chou developed the first 3-D printing device for tissue engineering in 1995. At that time, any commercial 3-D printing device 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.



2) In Vivo Studies of Bone Tissue Engineering

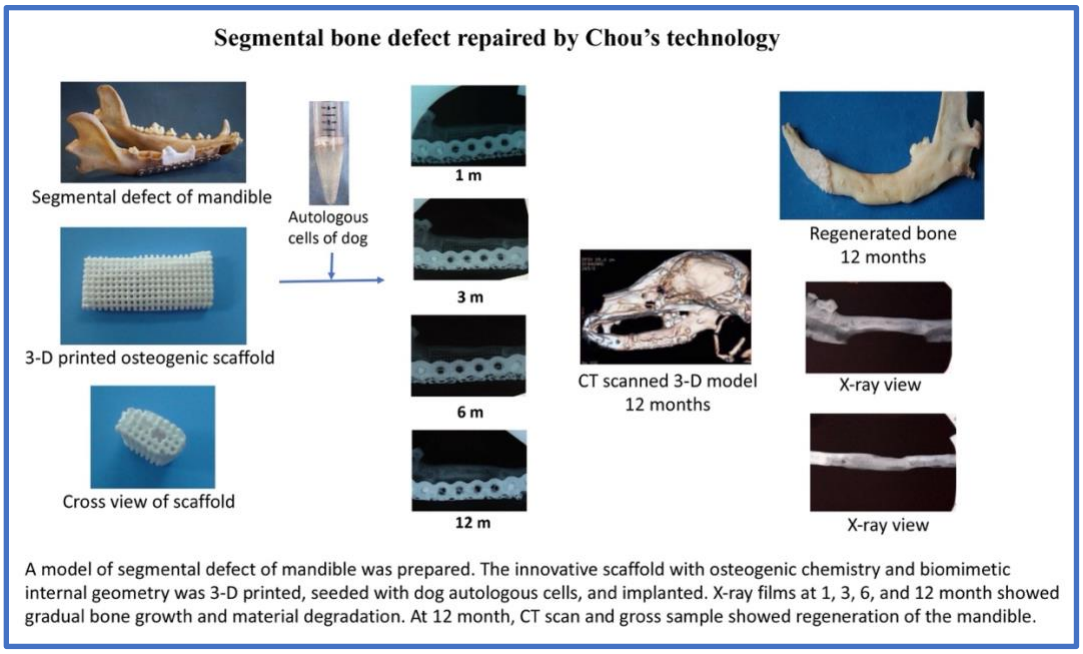
a) The first engineered human bone tissue

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 donner 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.

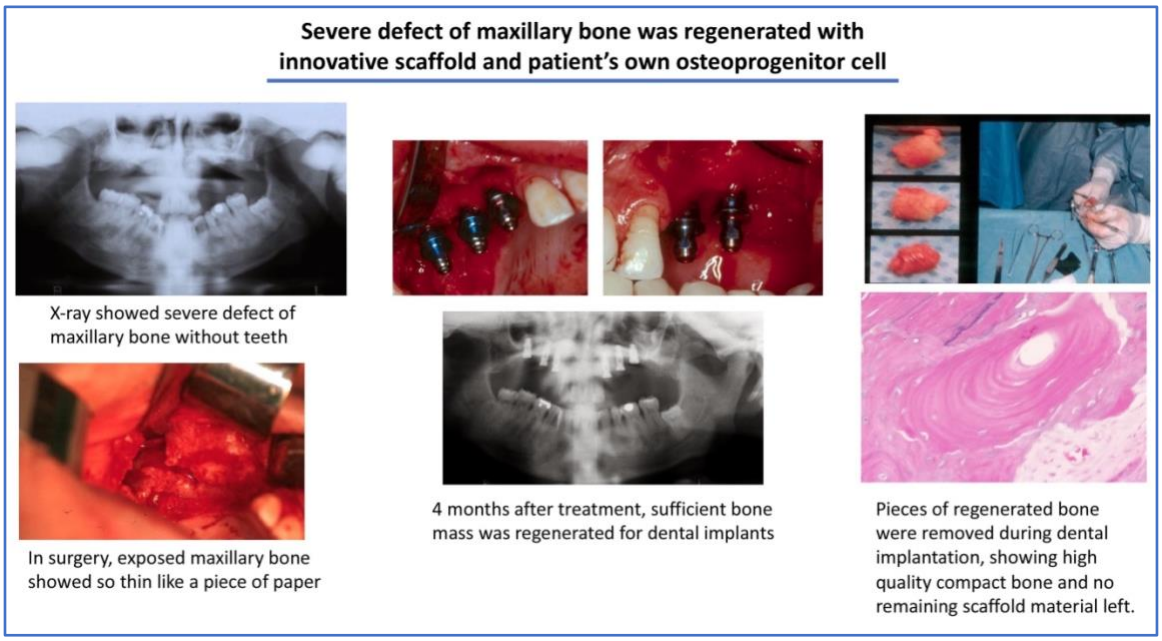
OSTEOBONE™ is a series of innovative products developed by Dr. Chou, covered by 19 patents. OSTEOBONE™ has successfully treated over fourteen thousands of patients with various kinds of bone defects including segmental bone defects, genetically inherited bone defects, server bone fractures, donor site 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.

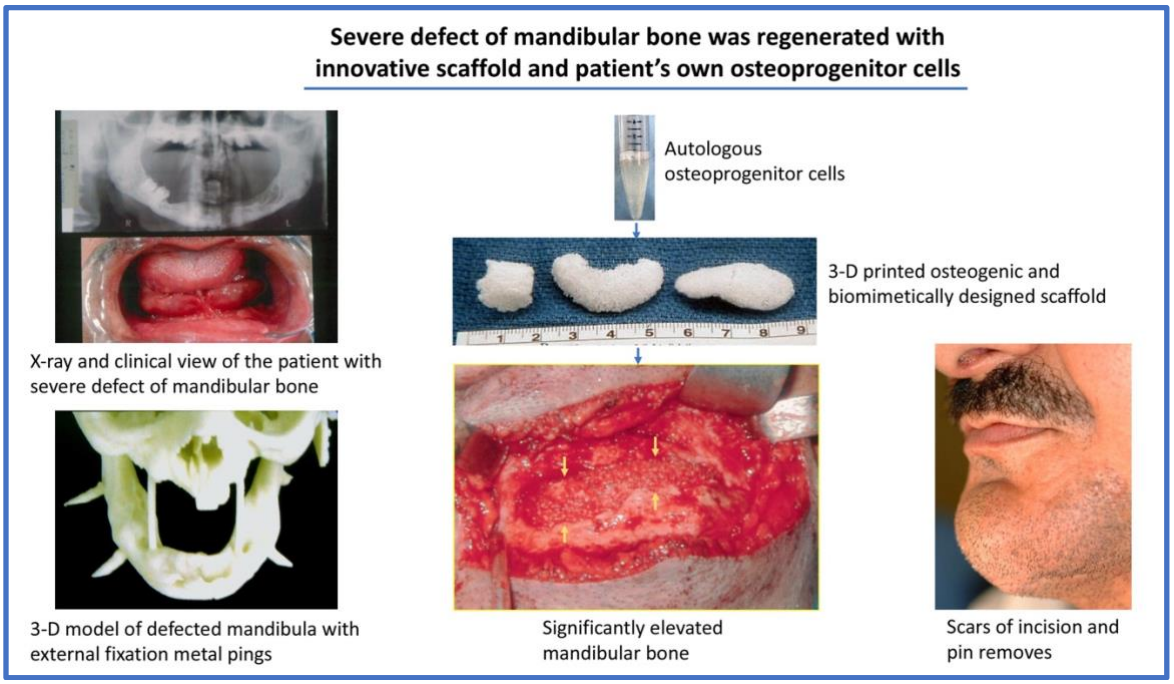
This patent of 26 years of age suffered with benign dominant osteopetrosis with significantly atrophy of his maxilla without posterior teeth. After his maxillary bone was regenerated, dental implants were placed with full function. Now, this patient has been followed up for over 25 years. All implants 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

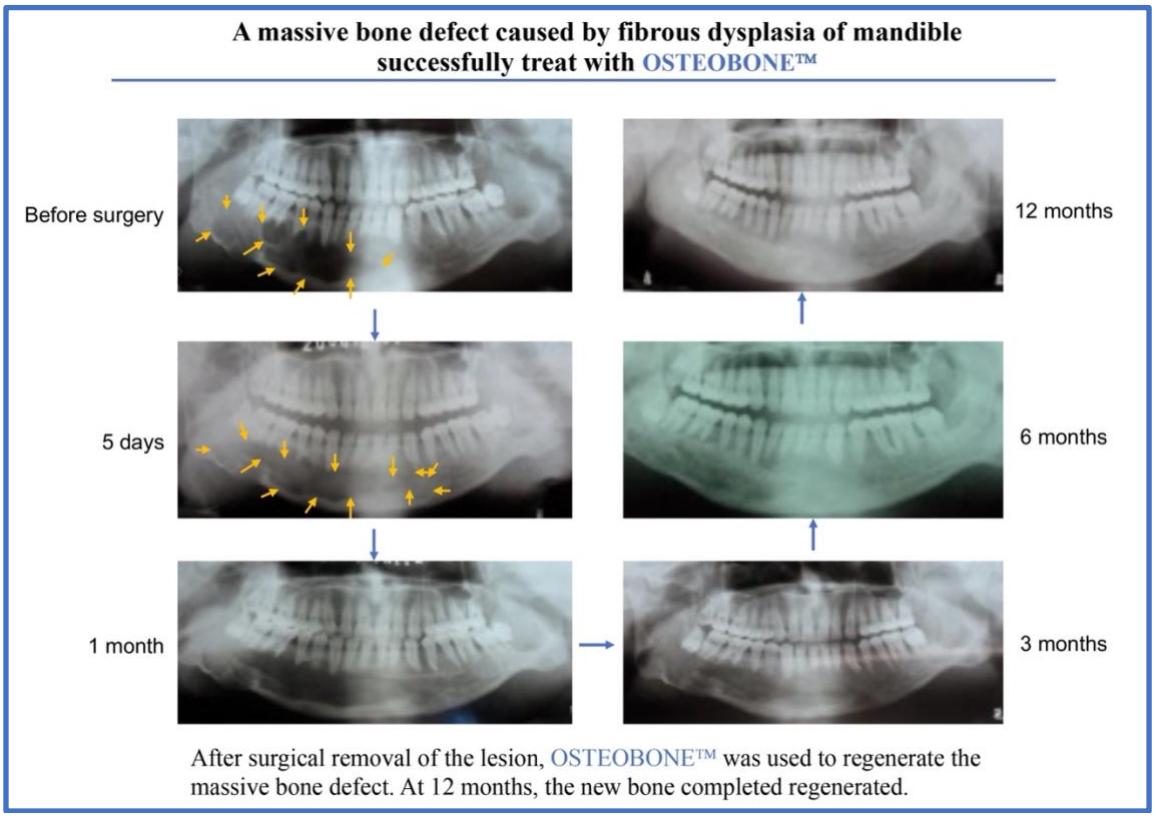
This was the second case successfully treated by Dr. Chou's inventive technologies of osteogenic materials, biomimetic design of scaffold internal geometry, 3-D printing device and patient's autologous osteoprogenitor cells under the IRB approval in 1999.

This patent 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)*

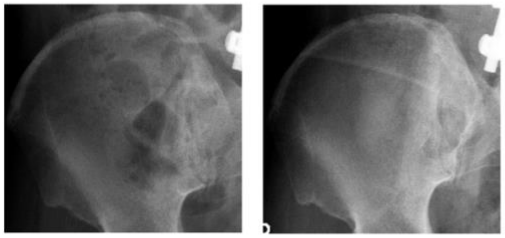


d) Bone donor site repaired with OSTEOBONE

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)*

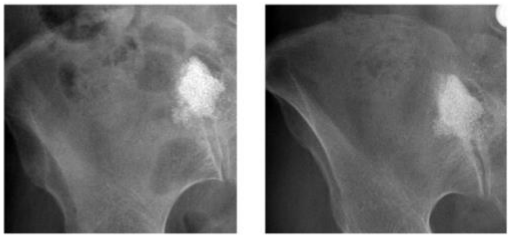
Comparing OSTEOBONE™ with HA in treating iliac ridge donor site defects

Case A - treated with OSTEOBONE™



1 week after surgery | 6 months after surgery

Case B - threated with HA

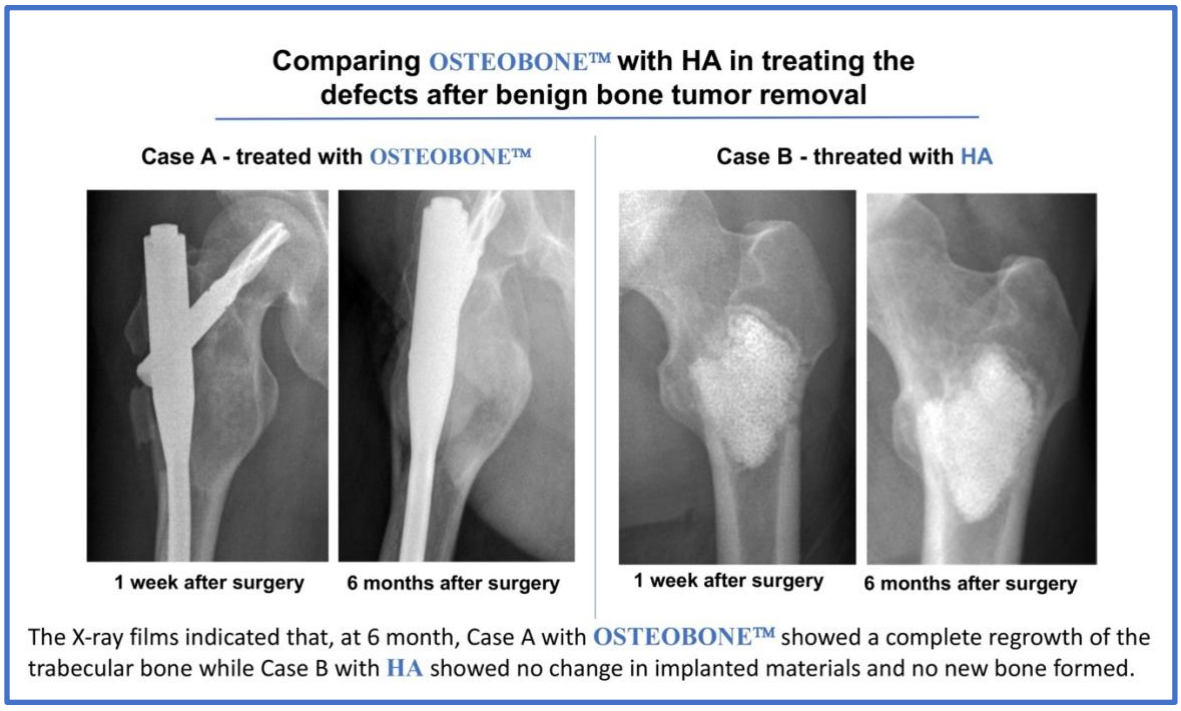


1 week after surgery | 6 months after surgery

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

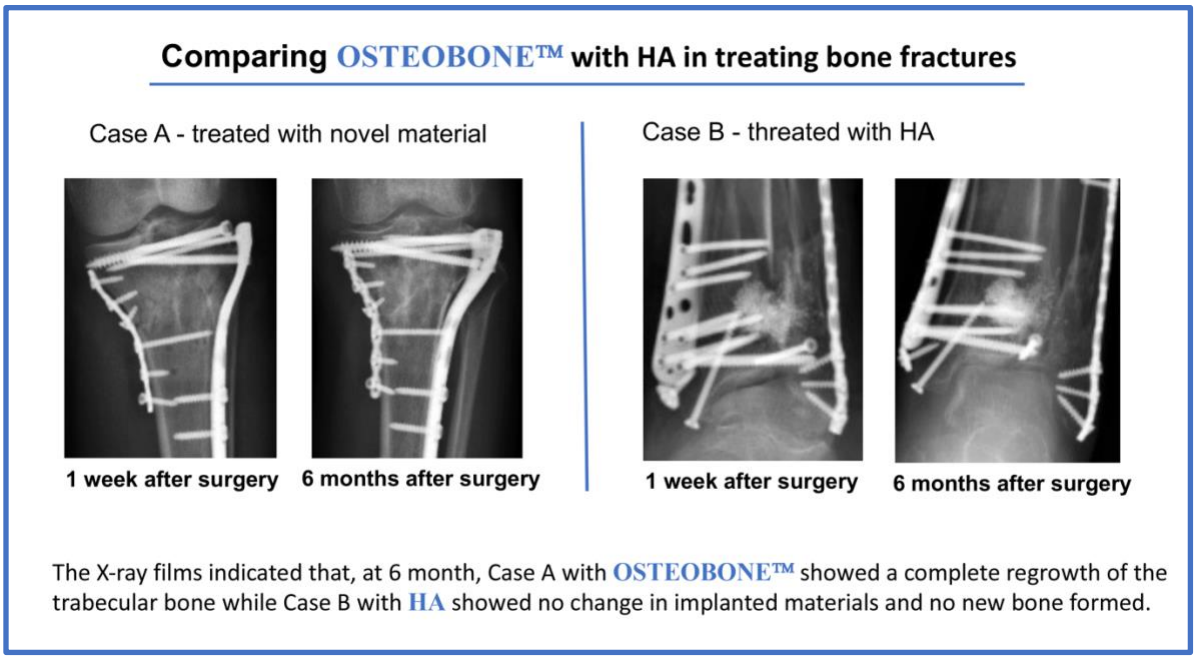
e) Bone defect after benign bone tumors treated with OSTEOBONE

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)*



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)*



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±8.82) was higher than that of the control group (109.10±11.40); The change in the height of the distal alveolar ridge of the second molar (H) in the **OSTEOBONE** group (4.56±1.43) mm was higher than that in the control group (0.93±0.83) mm; the visual analog scale value was significantly smaller than that of the control group (2.00±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://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.
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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