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.

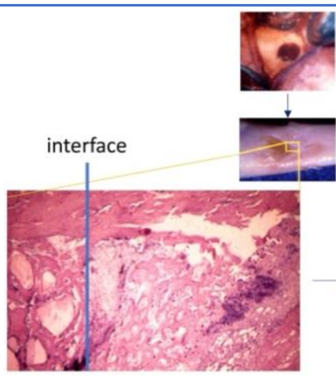


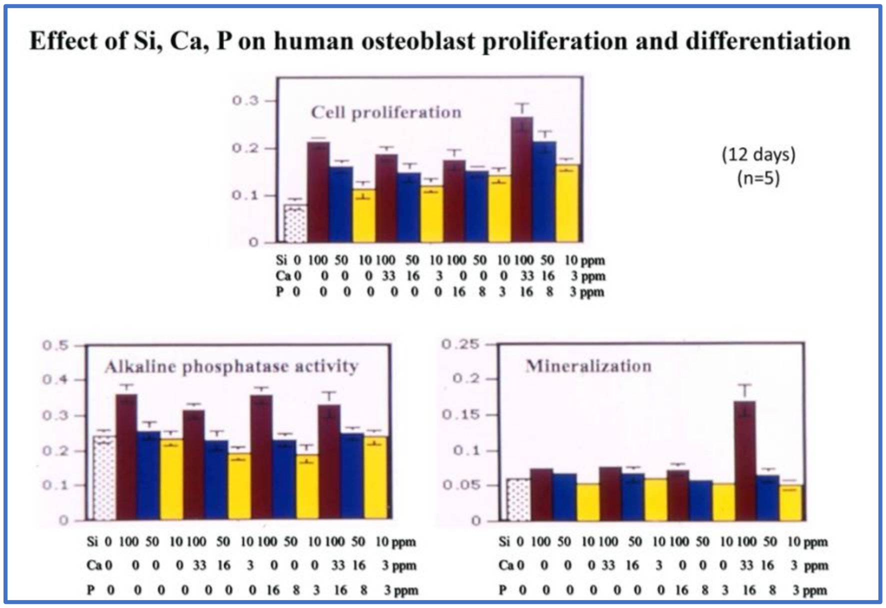
Table 3. Chemical Distribution at sol-gel Bioglass® Implant Interfaces (atomic %)

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.

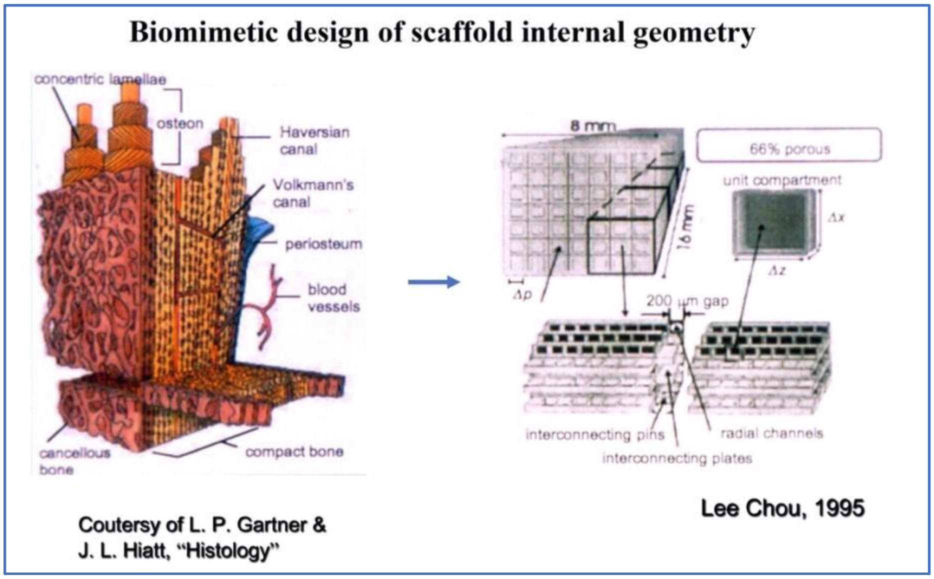
Chou, at el. Bioceramics, Vol. 11, 265-268, 1998.

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.



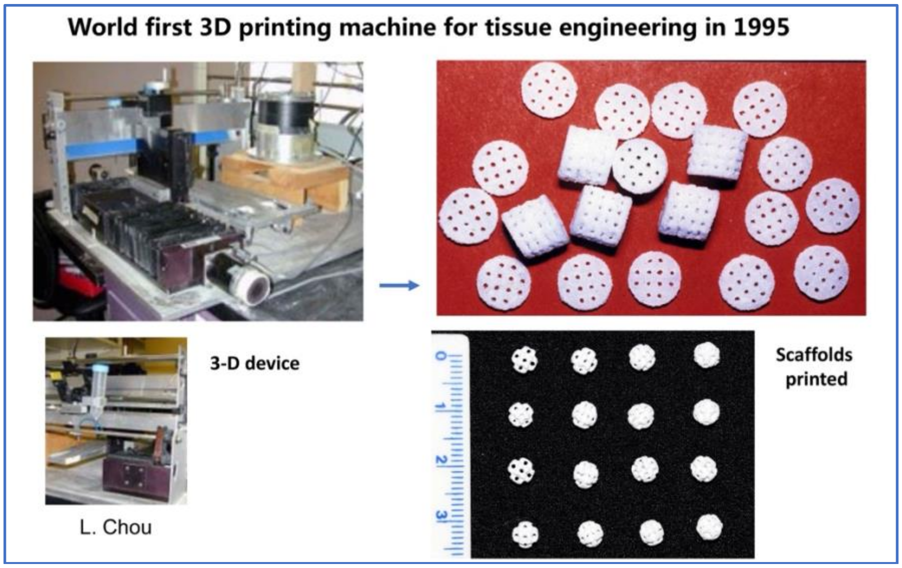
b) The first biomimetic scaffold designed by Dr. Chou for bone tissue engineering

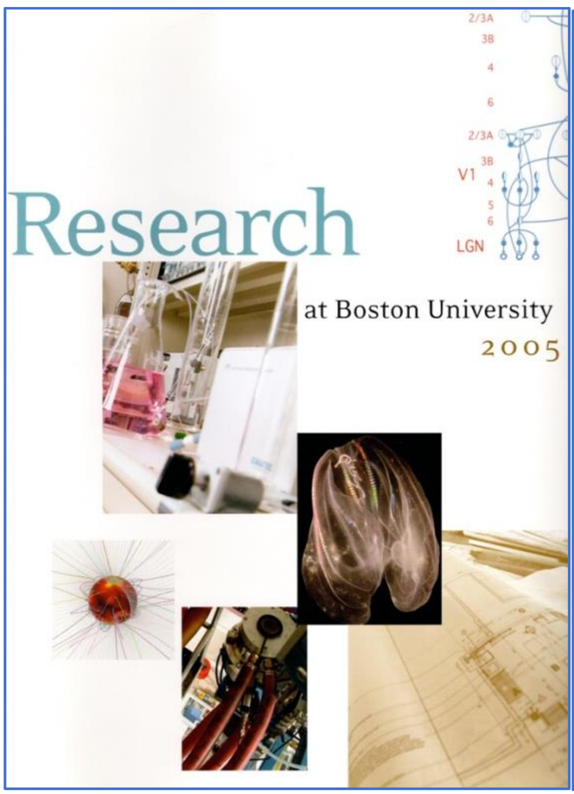
For many years, the scaffolds for tissue engineering were designed in a homogenized pattern with certain percentages of pores, which inhibited the cell distribution and blood vessel ingrowth evenly in the entire scaffold, resulting in failures of bone regeneration. Dr. Chou made the first biomimetic 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.





of healing will be done in one week. Although the research thus far has been conducted *in vitro* and several years of testing still 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 eats 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 Ketul 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, these 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 vessels 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.

Laisheng 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.

Ketul 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 100,000 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. Astoundingly, 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.

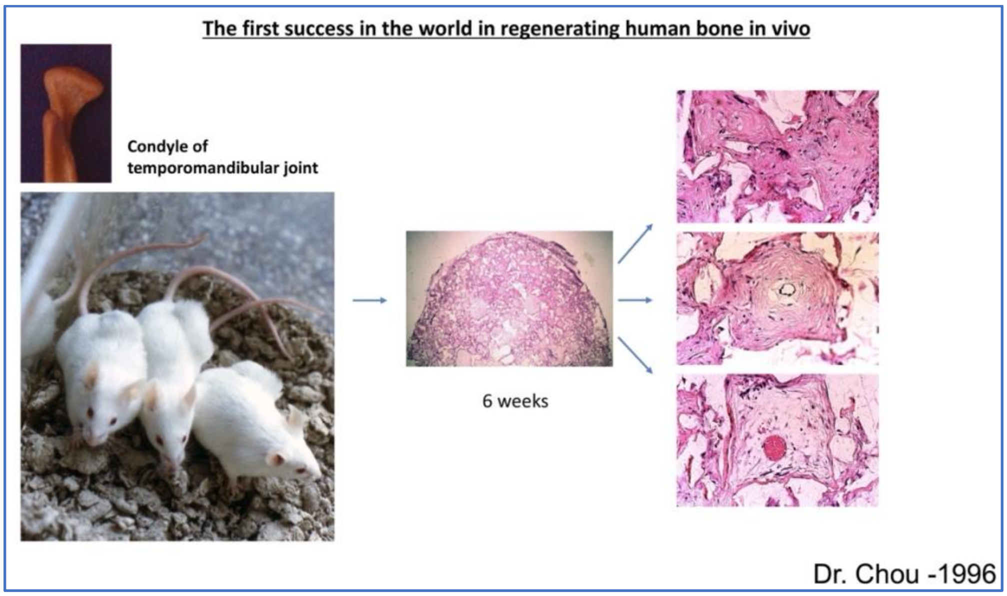
A man of many interests and talents, Chou also directs a project focusing on the mechanism by which the body takes in the AIDS virus. He also has been recognized for excellence in teaching—in 2002 he received BU's highest teaching award, the Metcalf Cup and Prize.

—Elana Hayasaka

2) In Vivo Studies of Bone Tissue Engineering

a) The first engineered human bone tissue

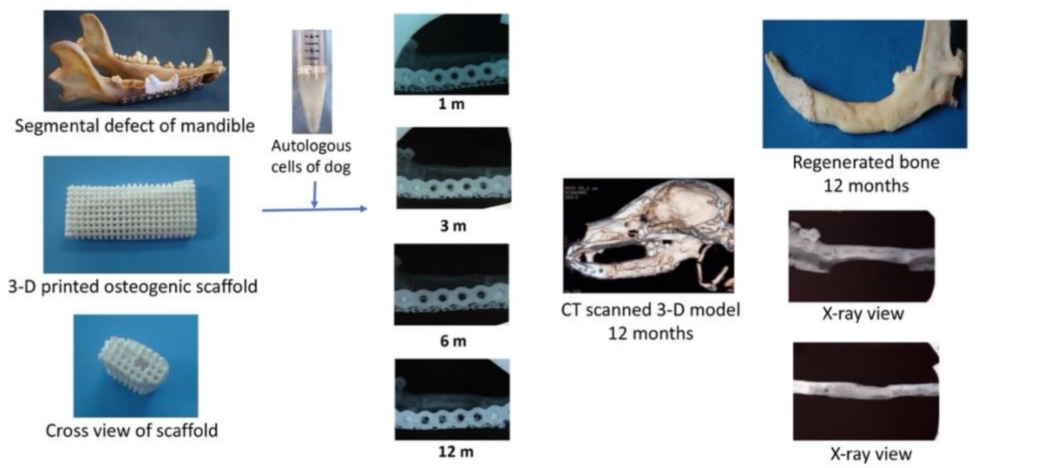
In 1996, Dr. Chou successfully engineered the human bone using the scaffold with his innovative osteogenic materials and biomimetic design of internal geometry. A large scaffold seeded with human osteoprogenitor cells derived from a healthy patient donner was implanted into SCID mice under Institution 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.

Segmental bone defect repaired by Chou's technology



A model of segmental defect of mandible was prepared. The innovative scaffold with osteogenic chemistry and biomimetic internal geometry was 3-D printed, seeded with dog autologous cells, and implanted. X-ray films at 1, 3, 6, and 12 month showed gradual bone growth and material degradation. At 12 month, CT scan and gross sample showed regeneration of the mandible.

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.

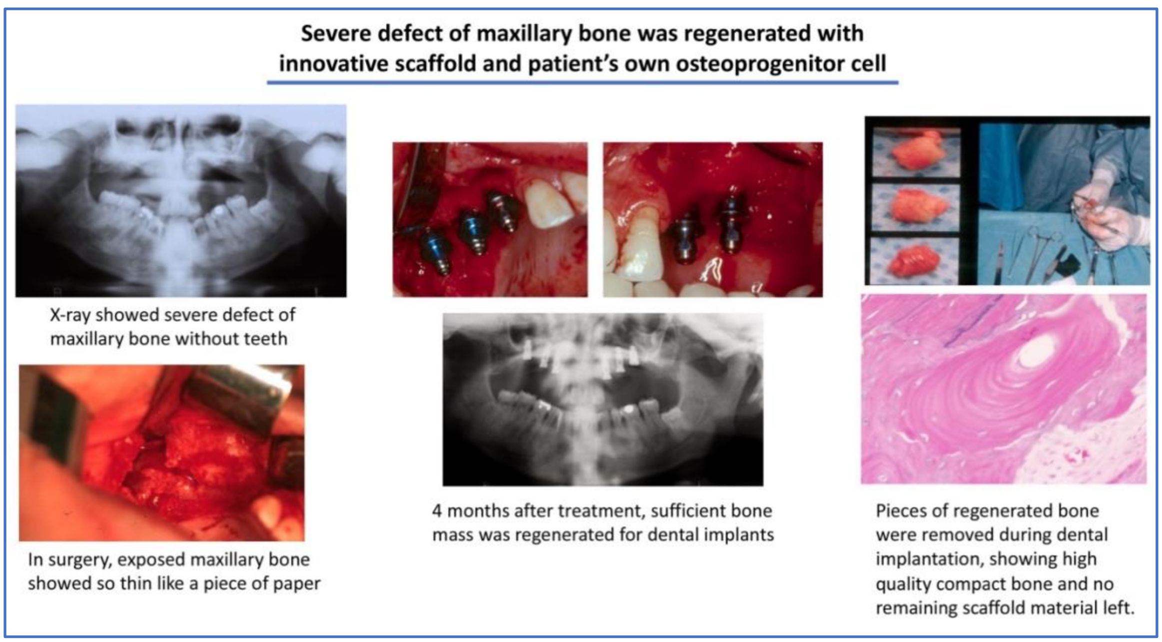
OSTEOBONE™ is a series of innovative products developed by Dr. Chou, covered by 19 patents. OSTEOBONE™ has successfully treated over fourteen thousands of patients with various kinds of bone defects including segmental bone defects, genetically inherited bone defects, server bone fractures, donor site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

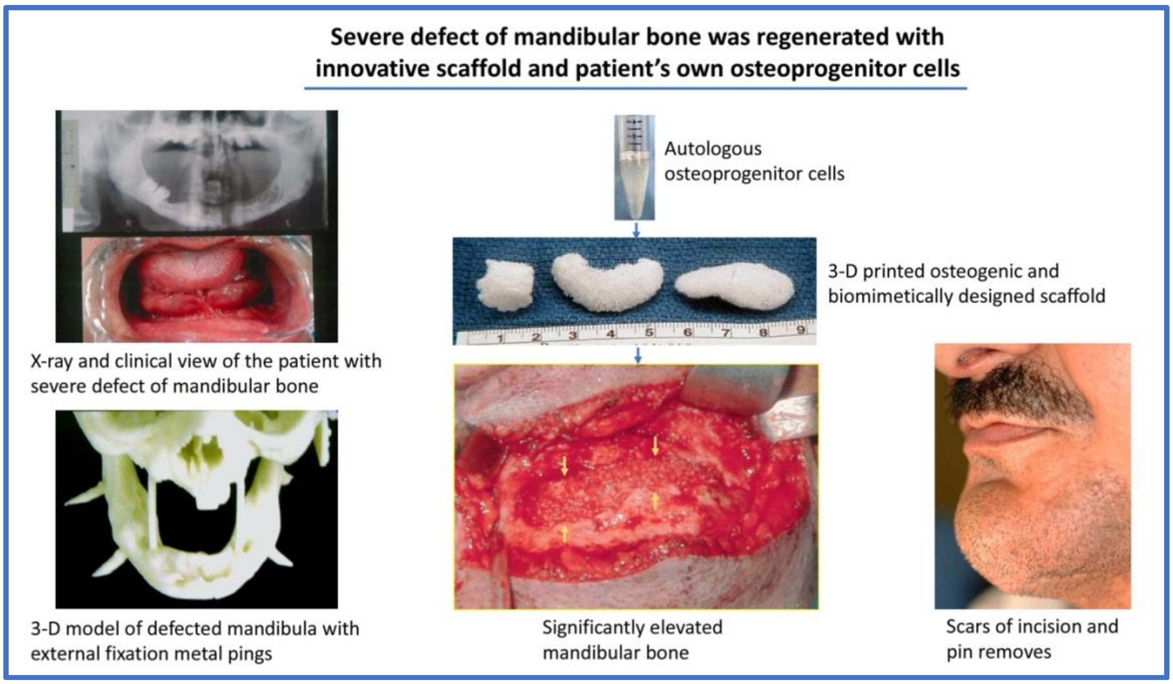
This patent of 26 years of age suffered with benign dominant osteopetrosis with significantly atrophy of his maxilla without posterior teeth. After his maxillary bone was regenerated, dental implants were placed with full function. Now, this patient has been followed up for over 25 years. All implants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

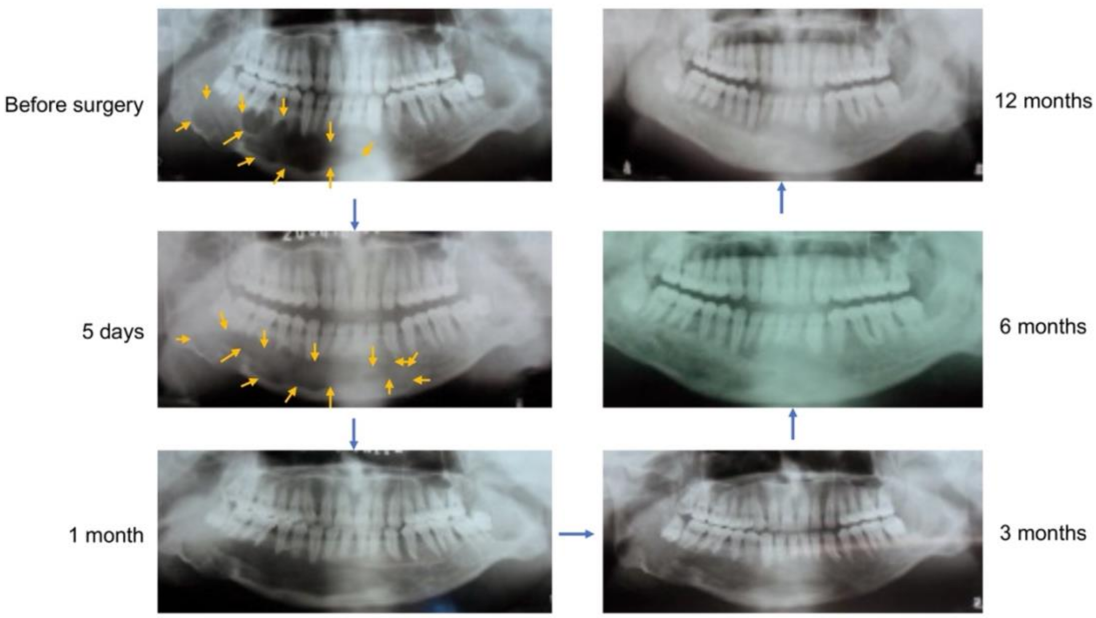
This patent of 45 years of age suffered with benign dominant osteopetrosis with significantly atrophy of his mandibular bone without any functional tooth. He also suffered with frequent fractures of his mandibula, so needed the external fixations with four external metal pins. After his mandibular bone 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)*

A massive bone defect caused by fibrous dysplasia of mandible successfully treat with OSTEOBONE™

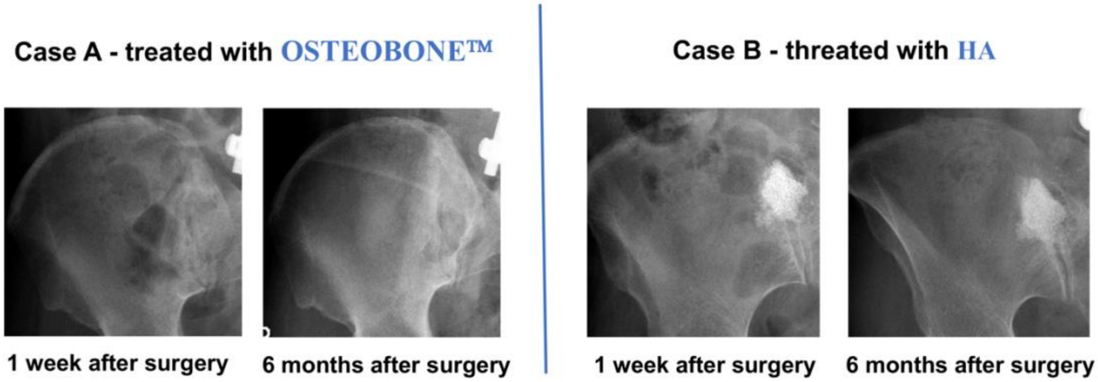


After surgical removal of the lesion, OSTEOBONE™ was used to regenerate the massive bone defect. At 12 months, the new bone completed regenerated.

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)*

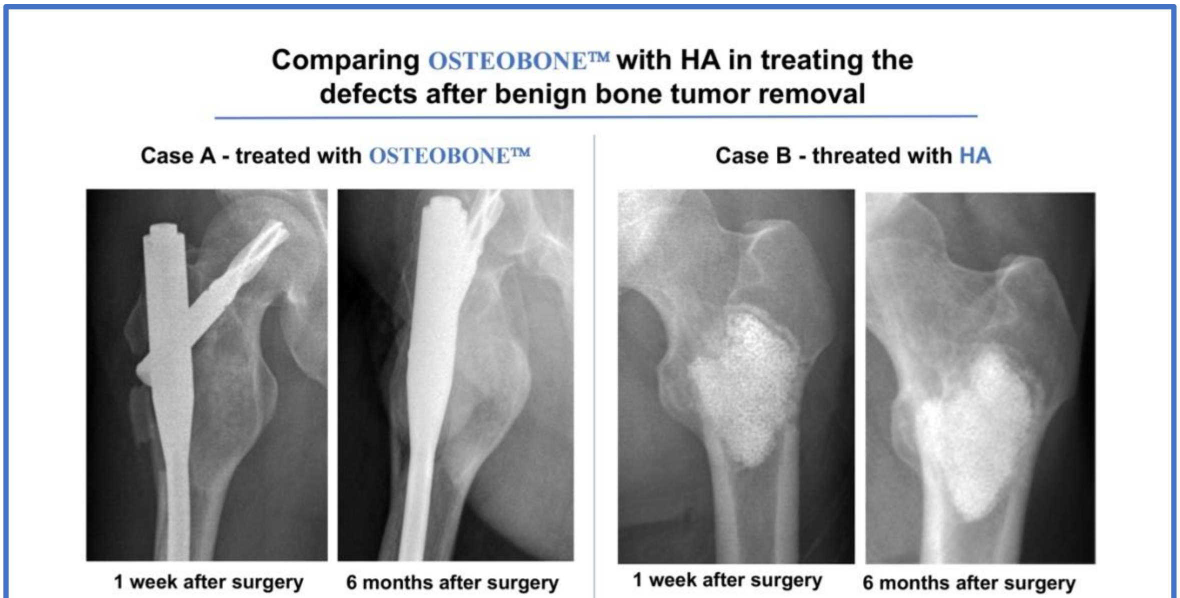
Comparing OSTEOBONE™ with HA in treating iliac ridge donor site defects



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**

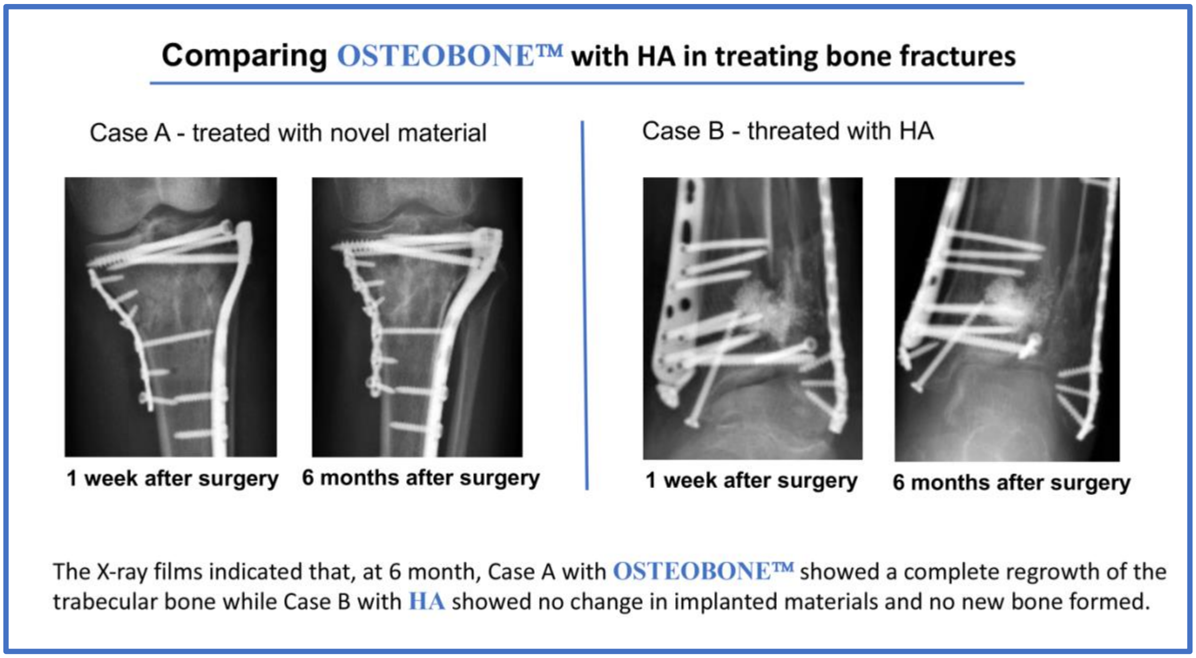
This patient was one of the large-scale clinical trials for treatment of defects after benign bone tumor removals with **OSTEOBONE** in comparison to the conventional treatment with hydroxyapatite (HA). The levels of material degradation and new bone formation are the measures of success. According to 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)*



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)*



5) Patents Covering the Invention and Production of OSTEOBONE

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

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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

To be added