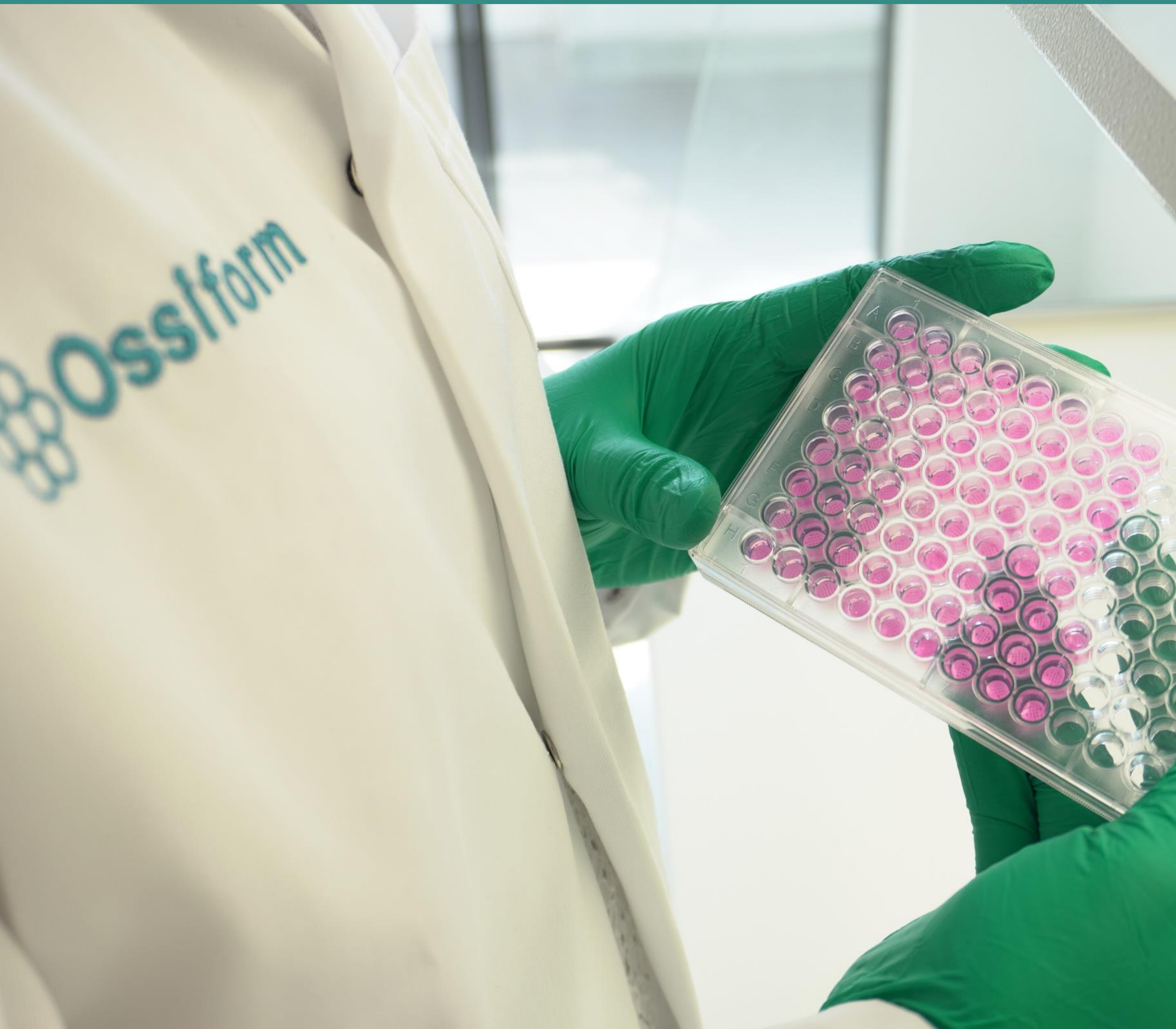
Ossiform

P3D Scaffolds

Product Promotion File



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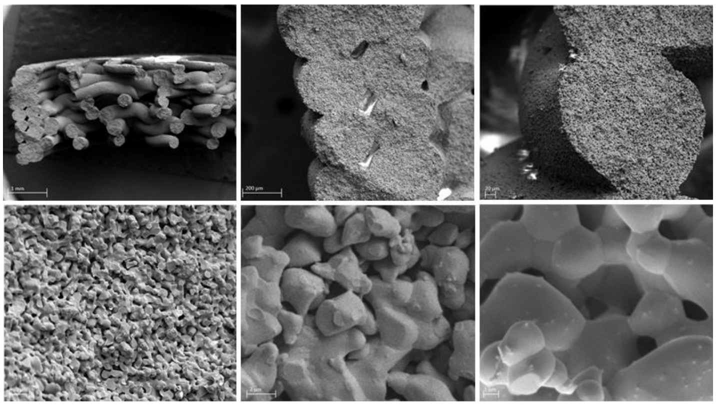
1. Introduction to P3D Scaffolds — porous 3D bone models based upon β-tricalcium phosphate

The study of 3D cell culture models has shown great potential in many applications including disease modeling, tissue engineering, and drug screening. Namely, because 3D cellular models offer an opportunity to better understand complex biology in a physiologically relevant context, where 2D models have not proven as successful.

Ossiform has developed P3D Scaffolds – a uniquely lifelike bone environment made from β-tricalcium phosphate (β-TCP) – using a patented 3D printing process. P3D scaffolds are three-dimensional (3D) cell growth support structures and tissue models made of natural, biocompatible materials. The lifelike bone models represent a reliable research model for studying bone disease mechanisms and testing new treatments such as drug- or cell therapy candidates. With the capacity to overcome the shortcomings and drawbacks of conventional 2D cellular models and animal models, the P3D Scaffolds show huge potential in research areas such as tissue engineering, disease modeling, regenerative medicine, drug screening, and stem cell and cancer research.

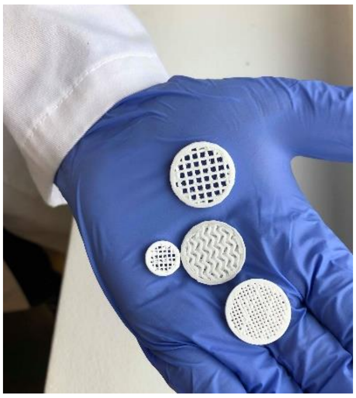
The structures are 3D printed with internal porosities to mimic the porous structure observed in human bones. In consequence, the lifelike models provide a more accurate replica of the bone composition and -stiffness, and therefore facilitates a more realistic growth. Such ceramic structures are difficult to create with traditional ceramic manufacturing techniques and even many 3D printing methods. P3D Scaffolds also enable the creation of good disease models, such as bone tumors.



SEM image of the microporous structure of P3D Scaffolds at different magnifications

As explained in more detail below, researchers can enhance and make their research more efficient with P3D Scaffolds, owing to the lifelike structures that secure a more realistic testing.

1.1. Physical properties and formats

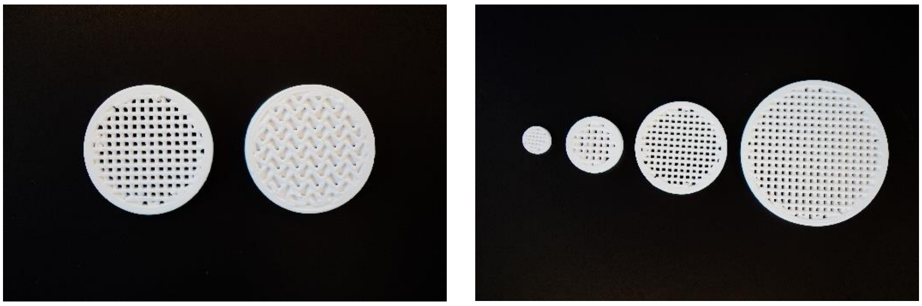


The P3D Scaffolds are provided in many different designs and sizes and may be fully customized to specific research needs to enable optimal testing conditions.

Four standard scaffold sizes are available: 5, 12, 20, or 30 mm in diameter, all with a height of 3 mm except the scaffolds with a diameter of 5 mm which have a height of 2 mm. The P3D Scaffolds are furthermore made with two different porosities, 3-400um and 6-800um. In addition, the scaffolds have several customizable features including design, pore size, height, and structure and density of the scaffold.

P3D Scaffolds offer the following customizable physical properties without any extra added cost:

- Choose between two designs (grid or gyroid).
- Choose if you want your scaffolds with a pore size between 3-400um or 6-800um.



A selection of P3D Scaffolds design variations and sizes.
1) Design: Grid or Gyroid 2) Size: 5, 12, 20, or 30 mm in diameter

If other physical properties are to be redesigned for a specially designed P3D Scaffold, an additional fee will be added to the price.

Our different infill types fulfill your different research needs. Do you need to monitor cell morphology close while the cells are still in culture? - Then go for our grid structure, which allows light to pass through the scaffold easily, thereby enabling you to perform regular checkups on your cells. Our data shows that cells will grow from the corners and in.

Is your research more focused on gaining a model system as natural as possible? Then our gyroid structure is just the right choice for you. Here you will get a randomized organization of the pores, resulting in a variable pore size, making regular checkups while in culture a bit trickier, but leaving you with a more accurate representation of the bone structure.

P3D Scaffolds are provided in the preferred quantity and size, either at a unit price dependent on scaffold size (Ø5mm, Ø12 mm, Ø20 mm, or Ø30 mm), or at a fixed price for a full plate solution.

1.2. Material

P3D Scaffolds are made from 3D printed β-TCP — a natural, biocompatible, and biodegradable material. β-TCP is the major mineral of the intercellular composite of human bones; this is why we want to model them using β-TCP, as this mineral contains only phosphate and calcium.

Because P3D Scaffolds do not contain any materials foreign to the body, the bone model resembles native bone tissue to a greater extent and is therefore well suited for in vivo animal trials as well as in vitro tests.

Since β-TCP is a widely used bone graft substitute, your research results will furthermore be directly relevant to many patients.

The scaffolds are available in regular and ultra clean β-TCP.

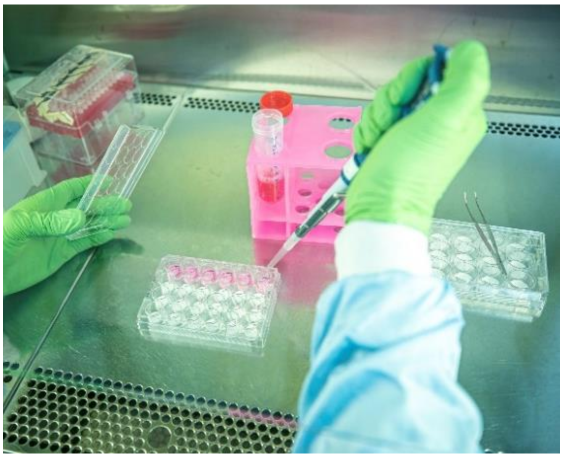
2. Benefits

2.1. Shortcomings of conventional methods

Until now, drug screening has primarily been performed in 2D with cell lines or in animal models, typically performed in mice. *In vivo* animal testing is currently required to document the safety and efficacy of a treatment in a living organism. However, animal models are time- and resource intensive and are not always a reliable way to predict how drug treatments will affect humans. Meanwhile, the traditional 2D cell culture models, widely used for *in vitro* research, do not accurately mimic the cellular environments of human organs or physiology. Namely, because the cells in our body do not grow flat in a monolayer.

2.2. The advantages of P3D Scaffolds

3D cell culture systems on the other hand, yield better and more reliable research models for studying the cellular environment as they maintain the cell-to-cell and cell-to-matrix interaction.



P3D Scaffolds provide a more accurate replica of the native 3D structures and material of human bone than other solutions. This, in consequence, facilitates a 3D cell growth and adhesion that resembles that of cells in their native environment. It also enables a better understanding of the complex biology in a physiologically relevant context when drug perfusion is uneven and where bacteria and cancer cells may hide in pores. Furthermore, while animal models are not always a reliable way to predict how diseases or drug treatments will affect humans, disease modeling and drug screening against human organs grown on scaffolds is, on the other hand, a very reliable method.

P3D Scaffolds increase effectiveness

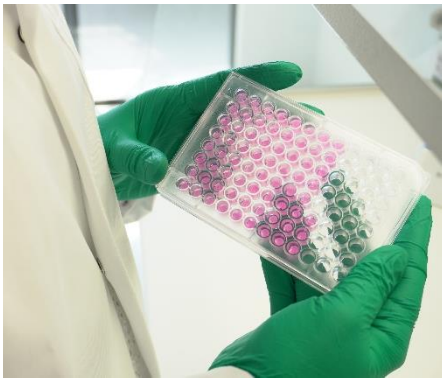
In consequence, the P3D Scaffolds can potentially increase researchers' effectiveness and efficiency. This is due to several benefits of the scaffolds:

- The more accurate, relevant, and reliable data deriving from the research models allows the researcher to fail or succeed faster.
- P3D Scaffolds may be used both *in vitro* and *in vivo*. Using scaffolds of the same natural material and porosity across research methods secures optimal conditions to obtain useful and relevant results.
- Finally, the accurate and reliable research models offer more relevant information and predictive data for in vitro and in vivo tests, and this is expected to reduce the use of the time- and cost-intensive animal trials.

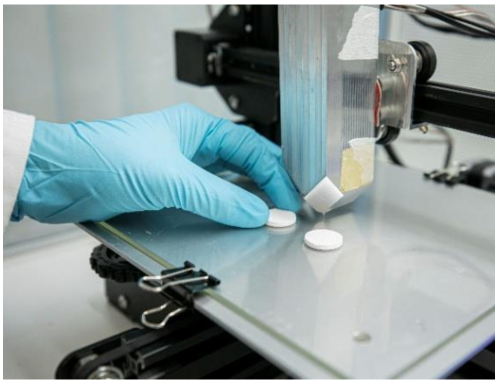
P3D Scaffolds may reduce animal testing

Accordingly, another important advantage of the P3D Scaffolds is the possible reduction in researchers' reliance on *in vivo* animal models to obtain relevant data. Reducing the use of animal models, owing to the relevant and predictive data from the 3D bone models, may consequently reduce the cost and time needed to develop new human therapeutics, and ultimately, get them into the clinic.

Importantly, this shift towards methods, such as P3D Scaffolds, that may reduce the use of animal models is in keeping with the goal of the US Environmental Protection Agency (EPA). The EPA aims to aggressively reduce animal testing, including reducing mammal study requests and funding 30% by 2025 and eliminate all animal testing by 2035. To this end, the agency awarded $4.25M to advance research on alternative methods to animal testing.



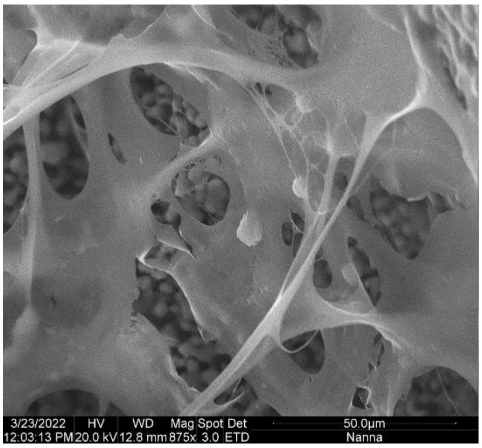
The shift from 2D to 3D technology is rapidly progressing, and new 3D cell culture systems and tissue modeling technologies like P3D Scaffolds have emerged in recent years. Still, Ossiform's natural bone scaffolds differentiate from other available solutions for bone research. That is, partly because P3D Scaffolds — unlike competing products — do not contain plastic and are therefore completely natural, resembling native bone tissue; and partly because the scaffolds are very well suited for animal trials as well as *in vitro* tests, as the P3D Scaffolds do not contain these unnatural foreign body materials.



In conclusion, the advantage of the P3D Scaffolds over other available solutions is rooted in the uniquely realistic and natural bone environment that they offer, yielding better, more accurate and reliable research, which in turn increases effectiveness.

3. Current and potential usages

3.1. Current usages of the P3D Scaffolds



At present, researchers are using the P3D Scaffolds to study the regenerative process of bones. The 3D-printed β-tricalcium phosphate implants from Ossiform were utilized as tissue engineering scaffolds in an *in vivo* study. The purpose of the experiment was to test and develop a system for non-invasive, non-destructive, and label-free monitoring of bone tissue development using novel spectroscopic techniques.

The material and customized shape of the scaffolds provided a clinically relevant system that allowed subcutaneous implantation in mice, while supporting osteogenic differentiation of mesenchymal stem cells.

3.2. Successfully tested and potential usages of the P3D Scaffolds

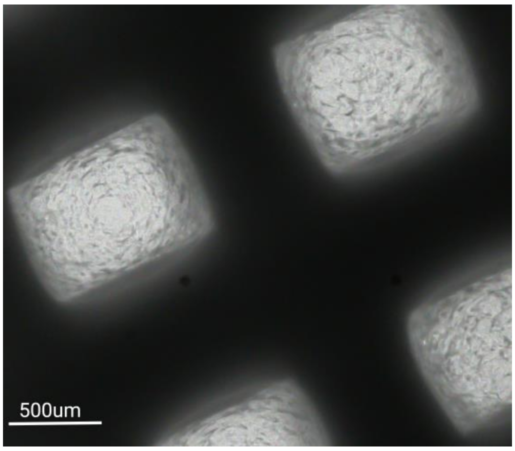
Various cells and pharmaceuticals can be added to the P3D Scaffolds to study how they interact with each other and with the bone within its pores. Potential usages include, but are not limited to, the examples mentioned in the following:

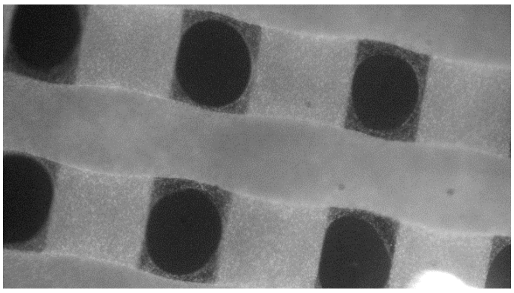
The P3D Scaffold enables the creation of good disease models, such as bone tumors, and is useful for studying diseases like bone metastasis, osteomyelitis, and osteoporosis. For example,

- Bone destruction in osteoporosis/arthritis can be imitated by seeding osteoclasts and macrophages onto the scaffolds.
- Osteomyelitis and the subsequent destruction of bone can be mimicked by adding bacteria and immune cells to the P3D Scaffolds. This enables you to study the mechanisms behind the bacteria's destruction of the hardened calcified bone as well as how the bacteria are able to evade the immune system and antimicrobial pharmaceuticals.
- The development of bone tumors and spreading of cancerous cells through the outer calcified bone matrix can be studied by seeding cancerous cells to the P3D Scaffolds (A potential use case for studying bone cancer pathology is presented in section 3.4).
- Mesenchymal stem cells or osteoblasts can be added onto the scaffolds to study how new bone develops in bone grafts.

Tested cell culture studies

Many different cells may be grown on the 3D printed structures. Ossiform has successfully grown the following cells on the structures:





Top: Light microscopy image of osteoblasts cultured on P3D Scaffolds for 21 days.
Bottom: Light microscopy with UV-filter and DAPI stain of osteoblasts cultured on P3D Scaffolds

- Stem cells including human mesenchymal stem cells for studying bone development.

- Cancer cells including oral carcinoma and lung cancer cells. These may be used to study cancer invasion and metastasis in bones, a frequent site of cancer metastasis and treatment of bone tumors using therapeutics.

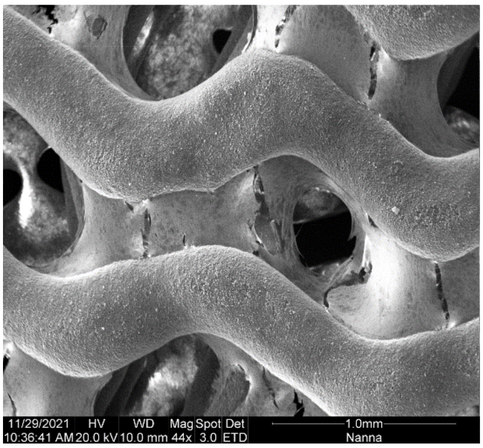
- Pathogenic bacteria including staphylococcus aureus. These may be used to study osteomyelitis, surgical site infections and implant biofilms, and prevention and treatment of these conditions using pharmaceutics.

- Environmental microorganisms including bacteria and fungi. These may be used to study the interaction between environmental microorganisms and inorganic 3D structures that mimic environmental structures like soil, rocks, sediment, and building materials.

- Resorption assays with human osteoclasts for studying osteoclast activation and resorption in various culturing conditions.

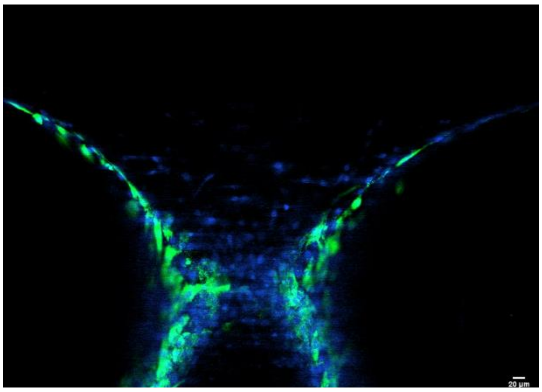
3.3. Assays and analytical methods compatible with the scaffolds

Many methods may be used for analyzing the biological activity on the 3D printed structures. Ossiform has successfully applied the following methods:



SEM image of ECM formed on P3D Scaffolds

- Raman spectroscopy.
- X-Ray Fluorescence (XRF).
- Scanning Electronic Microscopy (SEM).
- Energy-Dispersive X-ray Spectroscopy (EDX/EDS).
- Inverted light microscopy (through the pores).
- Micro-computed tomography (microCT) imaging.
- Nucleic acid extraction for PCR and Next Generation Sequencing (NGS).
- Staining assays such as an ALP staining assay or toluidine blue staining. The dye may be detached from the 3D structures after staining for quantification using absorbance.
- Fluorescence microscopy such as for human or bacterial cells that are labelled with Green Fluorescent Protein (GFP).
- Enzymatic assays such as the p-nitrophenyl phosphate-based assay for alkaline phosphatase activity.
- Confocal microscopy (LCSM)
- CellTiter-Glo 3D cell viability assay
- Live/Dead Stain
- Western Blot



Confocal microscopy image of osteoblasts (blue, DAPI) and their ECM (green, collagen type 1) on P3D Scaffolds

3.4. Use case: Bone cancer pathology

In the primary bone cancer field, there is no good model to understand this pathology. That is, because 2D cell lines fail to mimic the histoarchitecture of the primary bone cancer and the genetic landscape of the tumor.

The use of P3D Scaffolds would yield a better research model that could heighten our knowledge of bone cancer pathology. Namely, because the lifelike 3D structures maintain the cell-to-cell interaction and the cell-to-matrix interaction.
By mimicking the *in vivo* environment of tumors and the interplay between the tumor and the bone microenvironment, the 3D cell culture models enable the researcher to better understand these dynamic interactions. The models, created by the use of the P3D Scaffolds, thereby allow for accurate assessments of the efficacy of drug candidates in cancer patients as well as accurate predictions of patient outcomes.

In vitro	Tumoroid line	In vivo
P3D Scaffolds accurately mimic the histoarchitecture of the primary bone cancer, the genetic landscape of the tumor, and the interplay between the tumor and the host environment. A good disease model is thereby created.	Once a tumoroid line is established, you can use this line to test several drugs. The P3D Scaffolds yield accurate assessments of the efficacy of drug candidates in patients, allowing researchers to succeed or fail	According to the response to these drugs, you move on to animal trials, as the P3D Scaffolds can be used for both *in vitro* and *in vivo* research.

Use case: P3D Scaffolds for bone tumor modeling.

4. Studies and data

- Labiotech.eu. Can Biotechnology Reduce Animal Testing in Medicine? 2020-06-29: https://www.labiotech.eu/in-depth/animal-testing-bioprinting-organoids/

- EPA.gov. Administrator Wheeler Signs Memo to Reduce Animal Testing, Awards $4.25 Million to Advance Research on Alternative Methods to Animal Testing. 2020-10-09: https://www.epa.gov/newsreleases/administrator-wheeler-signs-memo-reduce-animal-testing-awards-425-million-advance

- Walther, AR., et al. "In vivo non-invasive monitoring of tissue development in 3D printed subcutaneous bone scaffolds using fibre-optic Raman spectroscopy." *Biomaterials and Biosystems* (2022): 100059.

- Jensen, MB., Dahl, SS., Bau-Madsen, N. "Porous 3D Printed Bioceramic Scaffolds induce Osteoblastogenesis in vitro." *104th Annual Meeting of the American Association of Oral and Maxillofacial Surgeons* (2022). See poster

- Thygesen, T. et al. "Comparison of off-the-shelf β-tricalcium phosphate implants with novel resorbable 3D printed implants in mandible ramus of pigs." *Bone* 159, 116370 (2022).

- Jensen, MB. et al. "Treating mouse skull defects with 3D printed fatty acid and tricalcium phosphate implants." *Journal of Tissue Engineering and Regenerative Medicine*. (2020): 1-11.

- Jensen, MB. et al. "Composites of fatty acids and ceramic powders are versatile biomaterials for personalized implants and controlled release of pharmaceuticals." *Bioprinting* 10 (2018): e00027.

- Slots, C. et al. "Simple additive manufacturing of an osteoconductive ceramic using suspension melt extrusion." *Dental Materials* 33.2 (2017): 198-208.

- Jensen, MB. et al. "The performance of a new generation of 3D printed and drug and stem cell loaded implants in vitro and in vivo." *DASCS2017 Stem Cell Conference* (2017). See poster

- Kwakwa, K. A. et al. " Engineering 3D models of tumors and bone to understand tumor-induced bone disease and improve treatments." *Current osteoporosis reports* (2017): 247-254.

4.1. Intellectual property

Patent status: The product is protected by one or more US, European, and/or foreign patents.

4.2. Disclaimer

The products are "For Research Use Only (RUO)" and should not be used for clinical purposes. Ossiform makes no other warranties, expressed or implied, including the implied warranty of merchantability and the implied warranty of fitness for particular purpose.

5. Marketing materials, resources, and other attachments

- **Technical Data Sheet:** View or download here
- **A4 Flyer:** Provided in PDF format or in high-quality print: View flyer
- **Website:** https://ossiform.com/ossiform-research-line.aspx
- **3DHeals article:** 3D Printing Bone: A Novel Way to Study Bone Diseases In Vitro https://3dheals.com/3d-printing-bone-a-novel-way-to-study-bone-diseases-in-vitro/
- **Protocols:** View or download here
- **Ossiform YouTube channel – Video protocols:** Visit YouTube channel
- **News From the Lab:** View latest news from Ossiform's laboratory
- **Publications:** P3D Scaffolds in scientific papers and posters
- **Blog post: How 3D cell culturing is revolutionizing the fields of disease modeling and regenerative medicine:** https://ossiform.com/news/technical-blog-posts/3d-cell-culturing-is-revolutionizing-the-fields-of-disease-modeling-and-regenerative-medicine.aspx

- **Blog post: How miniature tumors can be grown on P3D scaffolds for cancer research:** https://ossiform.com/news/technical-blog-posts/how-miniature-tumors-can-be-grown-on-p3d-scaffolds-for-cancer-research.aspx
- **Blog post: What are organoids and why are they important?:** https://ossiform.com/news/scientific-blog-posts/what-are-organoids-and-why-are-they-important.aspx
- **Blog post: How do bones function and what are they made of?:** https://ossiform.com/news/scientific-blog-posts/how-do-bones-function-and-what-are-they-made-of.aspx

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