OSTEOINK™ APPLICATION NOTE

1 OSTEOINK™ PRINTING USING 3DDISCOVERY®

3D-PRINTED Calcium Phosphate Scaffold

The present technical note provides guidance for the printing of OsteoInk™ calcium phosphate paste using the *3D*Discovery® tissue printer (regenHU Ltd, Villaz-St-Pierre, Switzerland). The generation of a three-dimensional (3D) bone-equivalent calcium phosphate scaffold in a layer-by-layer method is described.

OsteoInk™ is a highly osteoconductive biomaterial close to the composition of human bone. Dedicated for hard tissue engineering such as bone, cartilage or structural scaffold manufacturing, OsteoInk™ can be combined with regenHU's biomaterial portfolio (e.g. BioInk®) to create complex 3D tissue mimetic models.

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Maintenance of OsteoInk™

Check the OsteoInk™ non-sterile cartridge for signs of damage during the shipment. It can be transported, used and stored at room temperature for 3 months.

Additional Equipment and Reagents Required

- Cartridge adapter head (regenHU, Art. N°602 002 764)
- Needle DD-135N ; ID=0.61/G20 short (regenHU, Art. N°900 002 796)
- 5cc Cartridge adapter (regenHU, Art. N°900 005 166)
- Object slides
- Water
- 75% ethanol

OsteoInk™ Properties

The scaffold parameters are depending on the several factors:

- The mechanical properties of OsteoInk™ are dependent on the scaffold shape, scaffold size and porosity. The proprieties of the strand deposed by the printer are also strongly dependent on the needle parameters length and shape.
- The final mechanical properties are highly dependent on the post-printing hardening and washing process. The hardening process is activated post-printing by total scaffold exposition to a water containing fluid (e.g. water, cell culture media, humidity). The final mechanical properties can be modulated by the temperature of fluid exposition, exposition duration and physical properties of the fluid.

Within this application note we suggest a post-printing hardening exposed at 100% air-humidity at 37°C for a duration of four days.

2 OSTEOINKTM SCAFFOLD PRINTING USING 3DDISCOVERY®

BioCAD® Design Program

1. Design a macroporous circular scaffold printable on glass slides. With these settings, the size of the pores is about 600 µm in the X-Y direction, and 300 µm in the Z direction. The volumetric porosity is about 40 to 50%, depending on the strand thickness.

Figure 1 gives an overview of the design of the scaffold. Table I contains all parameters required to correctly build it.

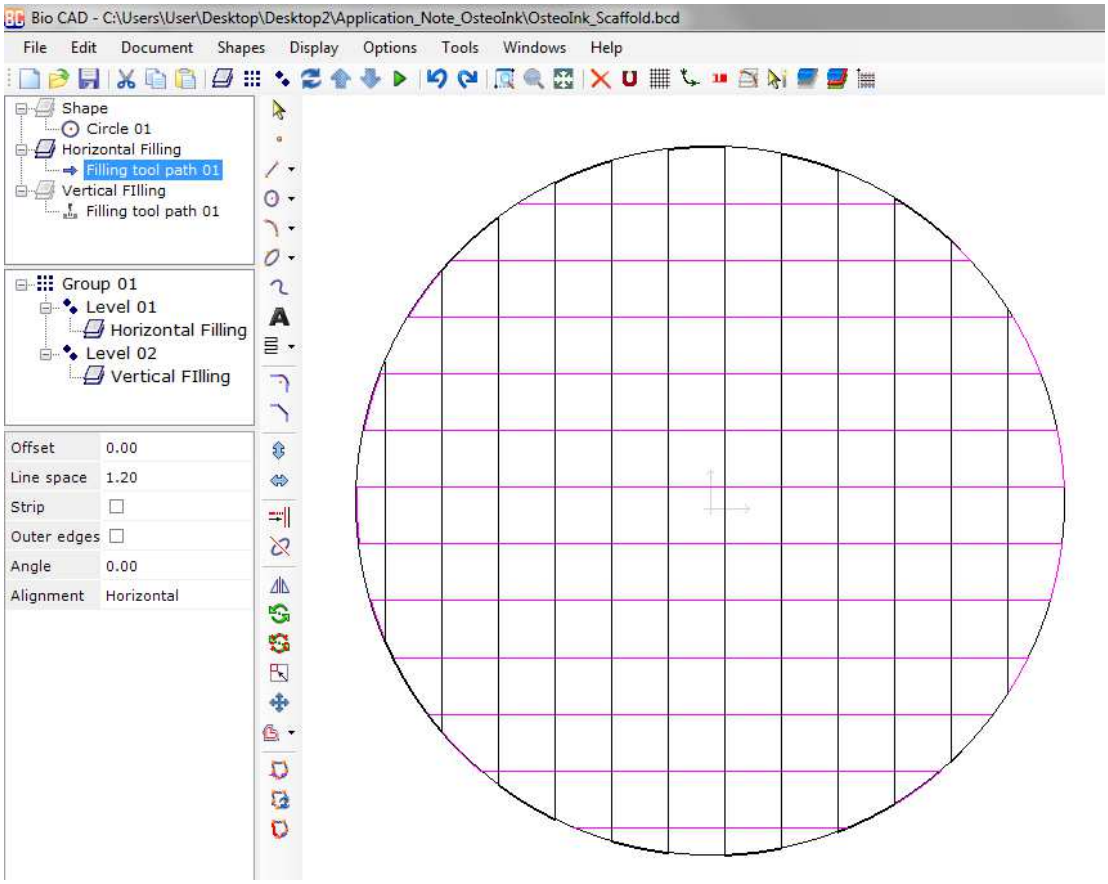

Figure 1: Overview of the design of the circular scaffold with BioCAD®

Table 1: BioCAD® parameters

Parameter	Value
Shape	Circle 7.5 mm radius
Filling	One horizontal and one vertical, 1.2 mm Line space
Group tree	Scaffold made with two levels in one group
Building high	10 mm
Feed rate	8 mm/s
Thickness	0.4 mm
Initial high	+0.6 mm (adds an offset to the substrate)

OsteoInk™ Handling

1. Switch on the *3D*Discovery® and initialize it. Start the HMI (Human Machine Interface) software.
2. Remove the upper and lower blue components of the cartridge, then assemble the OsteoInk™ cartridge with the DD-135N needle. Position them on the printhead by using a 5cc cartridge adapter. Connect the cartridge to its adapter head. Tighten the cartridge fixation screw. Refer to the *3D*Discovery® user manual for appropriate instrument handling

3D Discovery HMI Set-Up

1. Measure the needle length at the printhead set up with OsteoInk™.
2. Fix the glass slides on the *3D*Discovery® platform with the vacuum holder
3. Load the BioCAD® program for the design of a circular bone grafting macroporous scaffold model
4. Define the HMI print-head parameters as written in Table 2

Table 2: HMI print-head parameters

Parameter	Value
Material	OsteoInk
Needle	Needle DD-135N ; ID=0.61/G20 short (pink)
Pressure	3.5 -4.5 bar

5. Start the program run, print only 2-3 layers of OsteoInk™
6. Adjust and define the best pressure value for OsteoInk™ printing (3.5-4.5 bar) in order to obtain a porous scaffold with a conform shape.

Scaffold Printing

1. Fix the glass slides on the *3D*Discovery® platform with the vacuum holder
2. Start the program run, printing the whole structure
3. Check during the process that the strand thickness still allows a good porosity and a conform shape. If necessary, adjust the pressure (3.5-4.5 bar).

Hardening of the Printed Model

1. Remove gently the glass slide from the building platform
2. To reach its maximal stiffness, expose the OsteoInk™ printed model to 100% humidity at 37°C for four days.

3DDiscovery® Cleaning

1. Remove the cartridge from the printhead.
2. The needle is a consumable and can be thrown away
3. To re-use the cartridge in the future, plug the upper and lower blue components back on the cartridge and store it properly.
4. Remove the OsteoInk™ drops that might have been deposed on surfaces of the instrument with dry tissues.
5. Clean the surface with 75% ethanol.

3 QUALITY CONTROL

The quality of the OsteoInk™ model was tested after its preparation by printing and hardening.

- The scaffold model is printable at the above defined printing parameters
- The scaffold model is reaching its maximal stiffness with the above defined parameters
- The scaffold model has a macroporosity that allows a migration of living cells into it. The size of the pores is about 600 µm in the X-Y direction, and 300 µm in the Z direction. The volumetric porosity is about 40 to 50%, depending on the strand thickness.
- The scaffold model has a shape corresponding to the model designed with BioCAD®

InFigure 2 the model described above is showed before hardening.

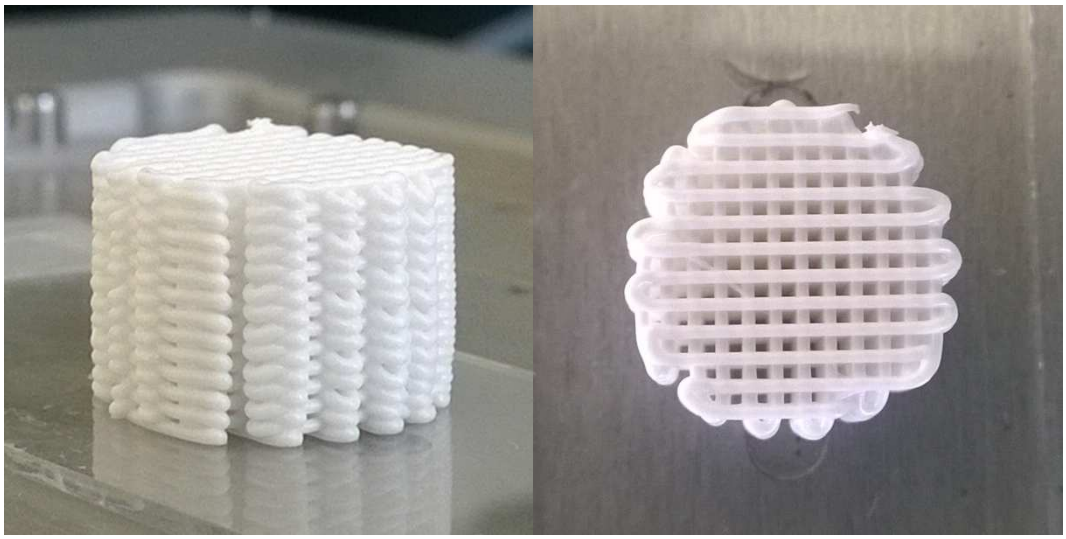

Figure 2: Detail view of the porosity of the bone-grafting scaffold model directly after the printing

Figure 3 shows the cut model after hardening. As expected there is a 3D porosity.

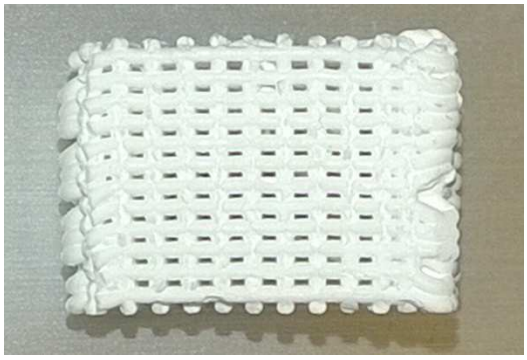

Figure 3: Detail view of the porosity of the bone-grafting scaffold model after hardening