Lecture 24-25

BT 636
Tissue Engineering and Regenerative Medicine
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Scaffold fabrication techniques

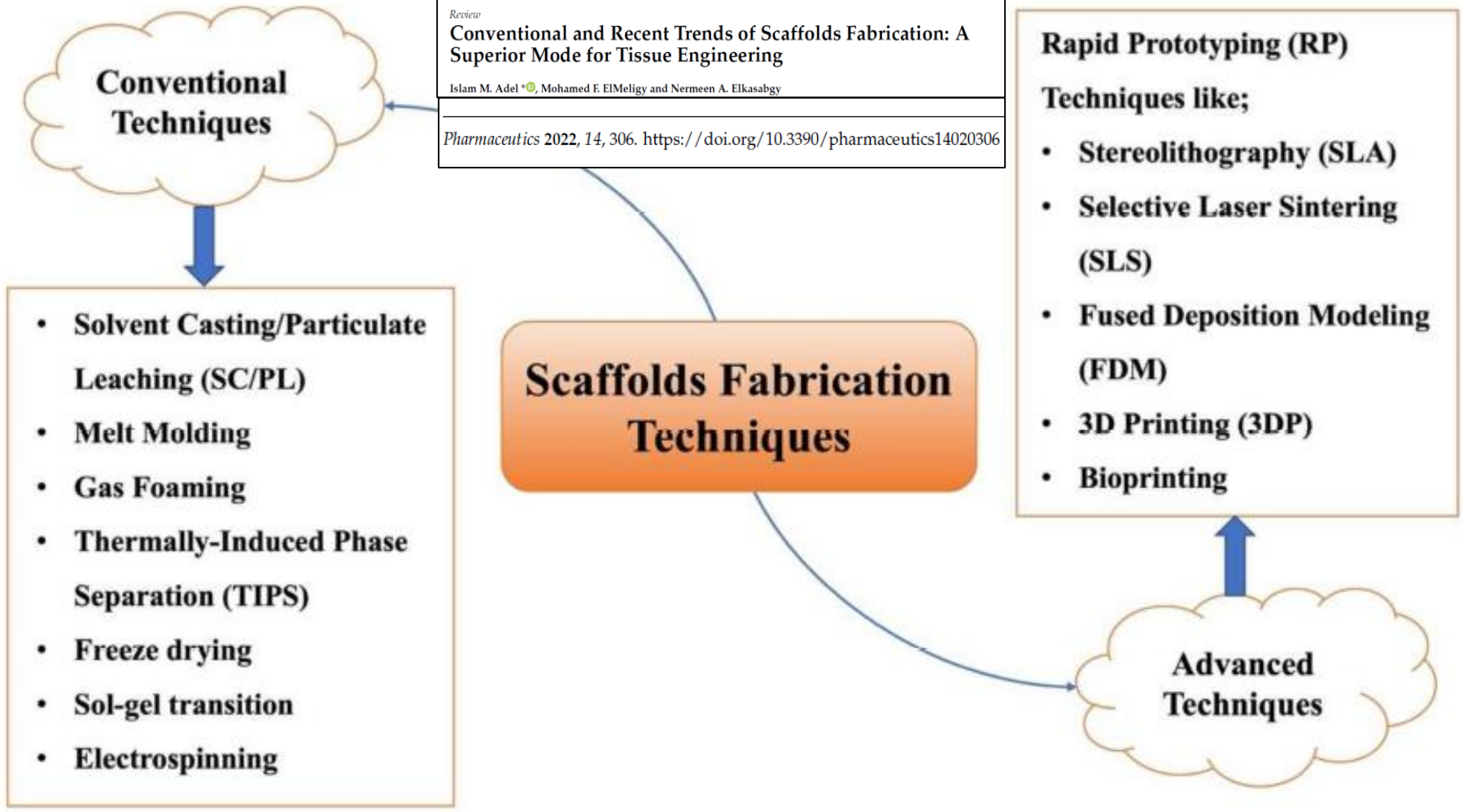


Figure 4. Classification of the numerous techniques that can be used in scaffolds fabrication into conventional and advanced techniques challenges and benefits of any of the mentioned techniques should be addressed prior to the scaffold fabrication to maximize patients' benefits.

Rapid Prototyping (RP)

- RP (referred to as solid free-form fabrication; SFF) techniques are based on computer-aided design (CAD) programs that design and construct scaffolds in a layer-by-layer, reproducible, and completely controlled manner.

- RP methods are, in fact, additive manufacturing (AM) approaches since raw materials, being solid powders or liquids, are added and solidified in a layer-by-layer manner.

- Advantages offered by RP techniques include the shorter time needed to reach satisfactory prototypes as well as the reduced trial-and-error stage in scaffold design and construction.

- However, the toxicity of binder liquids imposes health restrictions, and poor resolution of the techniques (50–300 μm) limits their usage in constructs requiring fine microstructure features.

- RP techniques are either based on laser technology or assembly techniques, and they include the likes of stereolithography, selective laser sintering, and fused deposition modeling.

Stereolithography (SLA)

- SLA was the **first laser-based**, 3D printing-based technology to be introduced by **Charles Hull** in **1986**.
- SLA is a form of 3D printing technology used for creating models, prototypes, patterns, and production parts in a layer by layer fashion using photochemical processes by which light causes chemical monomers and oligomers to cross-link together to form polymers.
- In SLA, a photosensitive liquid resin is irradiated by a UV light beam and allowed to deposit and solidify over a moveable platform forming the first layer.
- Once completely solidified, the platform is lowered, and the process is repeated for several layers until the desired prototype is obtained. After the process is finalized, the uncured resin is washed-off, and the prototype is further treated via UV exposure to obtain a fully cured product.
- Although the process is easy and yields 3D constructs with fine details, the processing time increases dramatically as resolution increases.
- Plus, many of the solidified polymers are non-biodegradable and may present toxicity hazards.

- Le Guéhennec et al. fabricated calcium phosphate-based pellets with or without HAp using SLA and evaluated them for their in vitro biocompatibility with MG-63 osteosarcoma cell lines and, for their in vivo osseointegration.
- In vitro findings confirmed the noncytotoxicity of the fabricated pellets; furthermore, the in vivo testing revealed the close contact (adhesion) between the pellets and the new bone tissue with excellent osseointegration.

Stereolithography (SLA)

- Stereolithography – more commonly referred to as SLA 3D printing – is one of the most popular and widespread techniques in the world of additive manufacturing.

- It works by using a laser source to harden liquid resin that is contained in a reservoir to create the desired 3D shape.

- In a nutshell, this process converts photosensitive liquid into 3D solid plastics in a layer-by-layer fashion using a laser and photopolymerization.

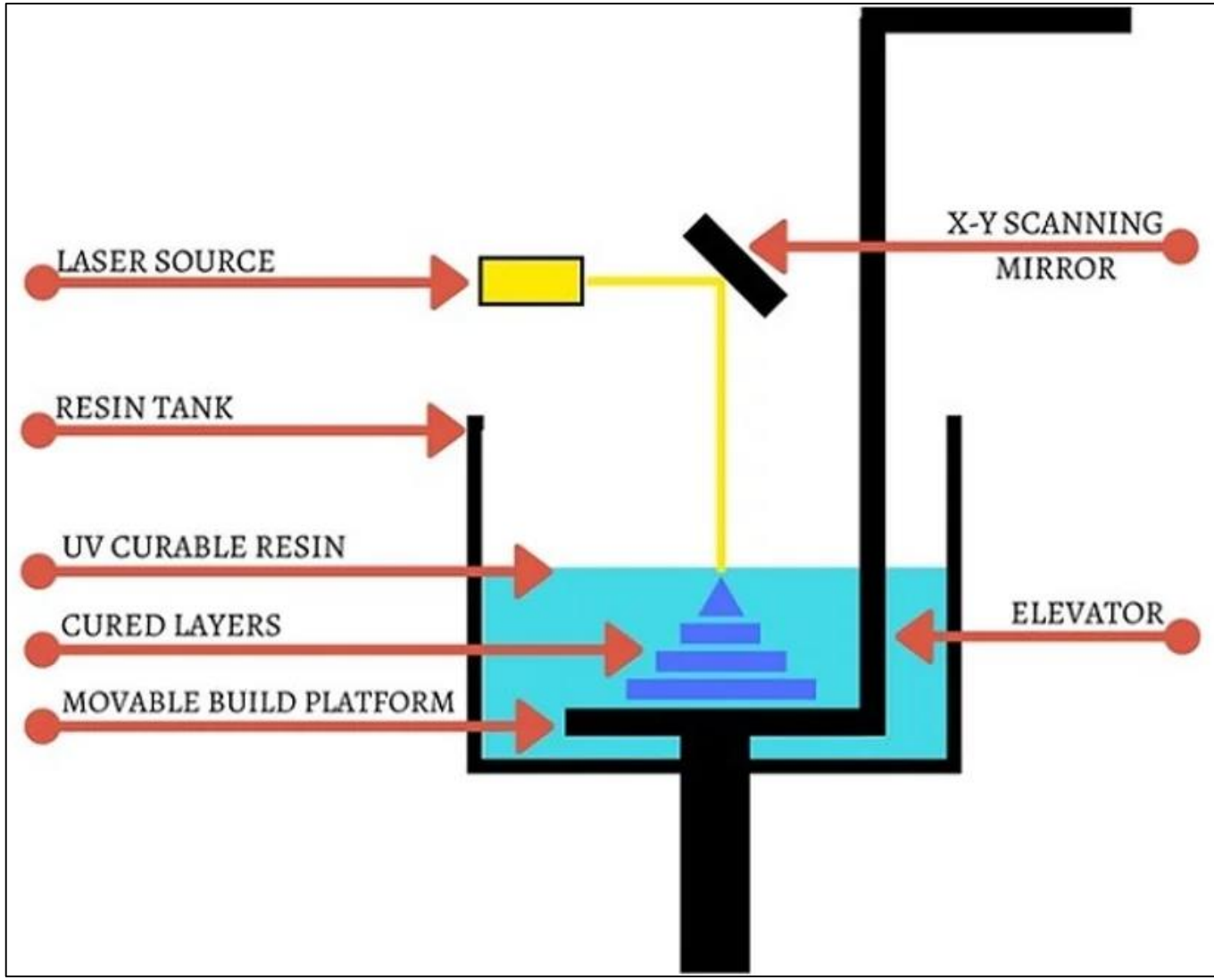
- SLA is one of three primary technologies adopted in 3D printing, together with fused deposition modeling (FDM) and selective laser sintering (SLS). It belongs to the resin 3D printing category.

- A similar technique that is usually grouped with SLA is called digital light processing (DLP). It represents a sort of evolution of the SLA process, using a projector screen instead of a laser.

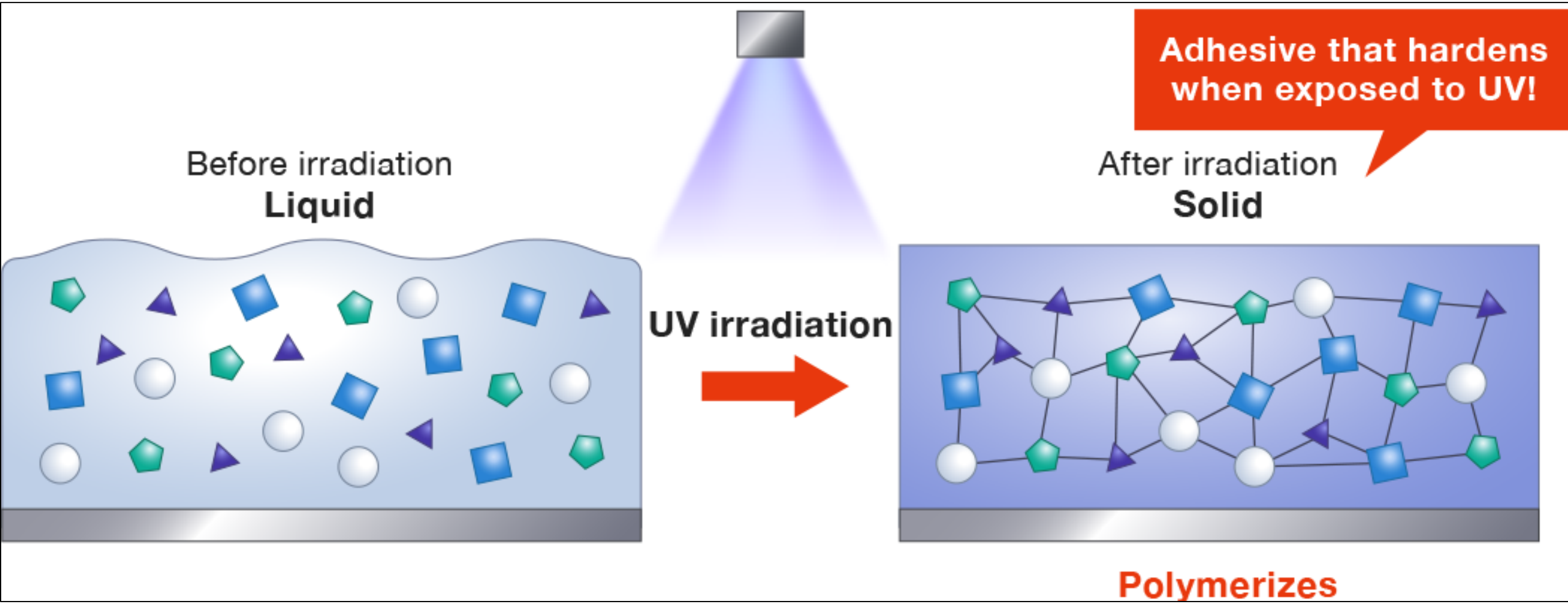
Stereolithography (SLA) - Components

- Every standard SLA 3D printer is generally composed of **four primary sections**:

- A tank filled with the liquid photopolymer: The liquid resin is usually a clear and liquid plastic.
- A perforated platform immersed in a tank: The platform is lowered into the tank and can move up and down according to the printing process.
- A high-powered, ultraviolet laser
- A computer interface, which manages both the platform and the laser movements

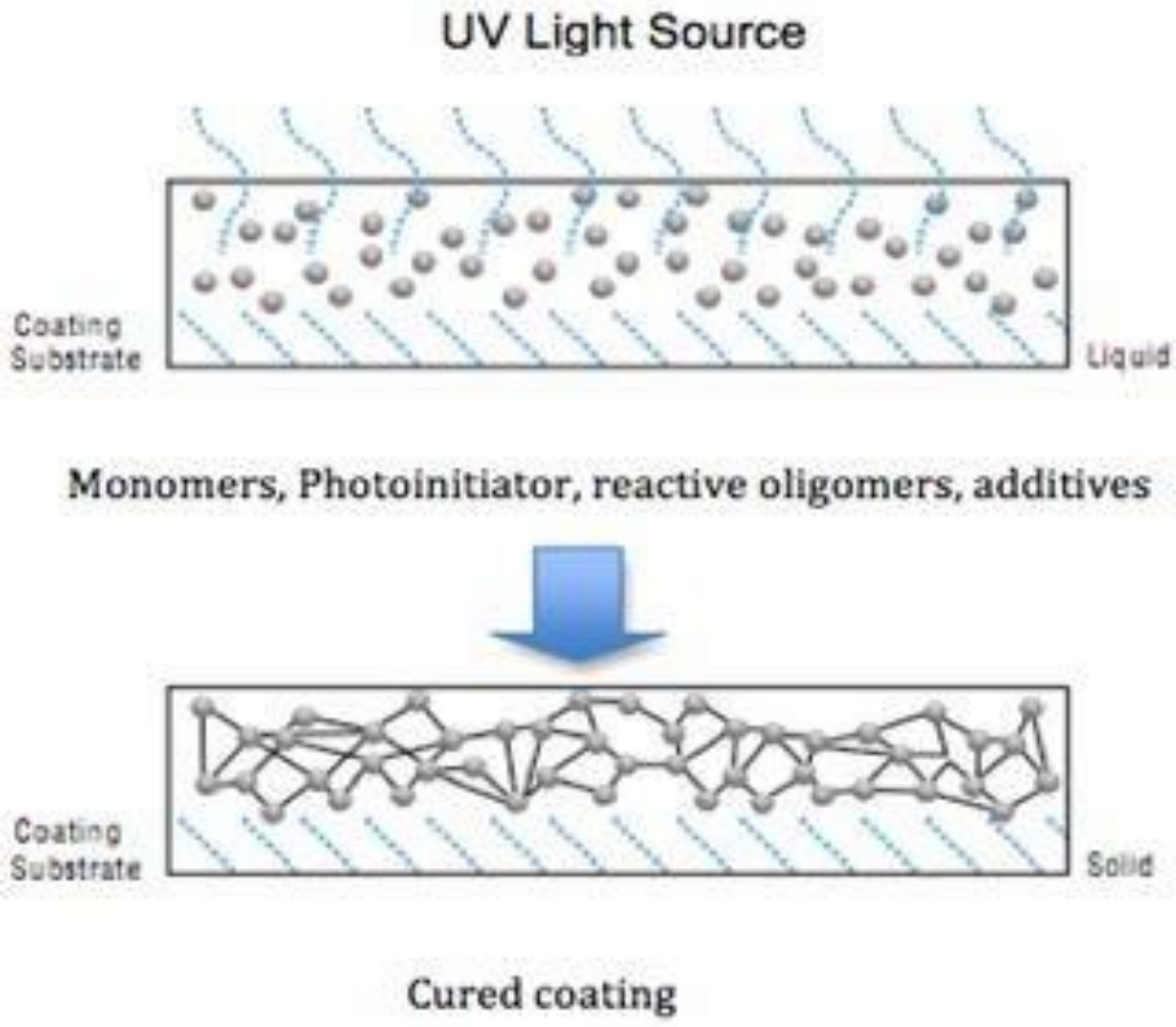


Stereolithography (SLA) – History



Stereolithography (SLA) – History

Figure 1 – UV Cure Polymerization



UV CURE REACTION SEQUENCE

Step 1 – Photoinitiator (PI) ***Lite absorption***

Step 2 – ***Activation*** of PI

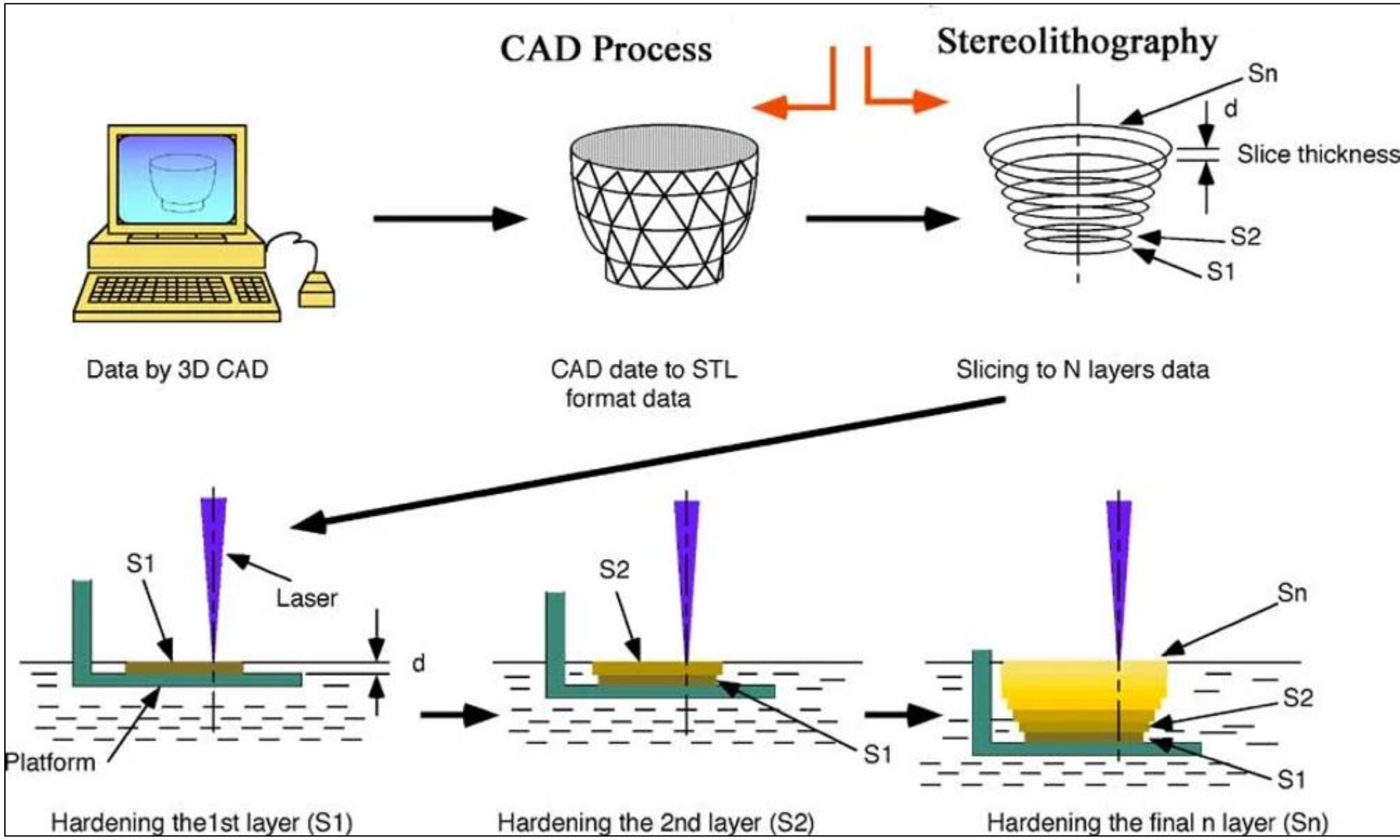
Step 3 – Chemical reaction of activated PI with monomers/oligomers to ***initiate polymerization***

Step 4 - ***Propagation*** of polymerization to cross-link coating

Step 5 – ***Termination*** of polymerization

Stereolithography (SLA) - How Does It Work?

3D CAD software (computer-aided design)



Process of stereolithography. Source: http://www.thagiwara.jp/rp-resin/IUPAC/iupac2000.html

Stereolithography (SLA) - How Does It Work?

- **Software**
- As is the case for many additive manufacturing processes, the first step consists of designing a 3D model through CAD software. The resulting CAD files are digitalized representations of the desired object.
- If they are not automatically generated as such, the CAD files must be converted into STL files. Standard tessellation language (STL), or “standard triangle language”, is a file format native to the stereolithographic software created by the Abert Consulting Group specifically for 3D Systems back in 1987.
- **STL files describe the surface geometry of the 3D object, neglecting other common CAD model attributes, such as color and texture.**
- The pre-printer step is to feed an STL file into a 3D slicer software, such as Cura. Such platforms are responsible for generating G-code, the native language of 3D printers.

- **SLA 3D Printing**
- When the process starts, the laser “draws” the first layer of the print into the photosensitive resin. Wherever the laser hits, the liquid solidifies. The laser is directed to the appropriate coordinates by a computer-controlled mirror.
- After the first layer, the platform is raised according to the layer thickness (typically about 0.1 mm) and the additional resin is allowed to flow below the already-printed portion. The laser then solidifies the next cross-section, and the process is repeated until the whole part is complete. The resin that is not touched by the laser remains in the vat and can be reused.

- **Post-Processing**
- After finishing the material polymerization, the platform rises out of the tank and the excess resin is drained. At the end of the process, the model is removed from the platform, washed of excess resin, and then placed in a UV oven for final curing. Post-print curing enables objects to reach the highest possible strength and become more stable.

Stereolithography (SLA) - Pros & Cons

- **Pros**
- SLA is one of the most precise 3D printing techniques on the market.
- Prototypes can be created with extremely high quality, with finely detailed features (thin walls, sharp corners, etc…) and complex geometrical shapes.
- Layer thicknesses can be made as low as 25 µm, with minimum feature sizes between 50 and 250 µm.
- SLA provides the tightest dimensional tolerances of any rapid prototyping or additive manufacturing technology: +/- 0.005″ (0.127 mm) for the first inch, and an additional 0.002″ for each additional inch.
- Print surfaces are smooth.
- Build volumes can be as high as 50 x 50 x 60 cm^3 without sacrificing precision.

- **Cons**
- Printing tends to take a long time.
- Steep slopes and overhangs require support structures during the building process. Such parts may potentially collapse during printing or curing phases.
- Resins are comparatively fragile and therefore not suitable for functional prototypes or mechanical testing.
- SLA offers limited material and color choice, usually offering black, white, grey and clear material. Resins are often times proprietary and therefore cannot be easily exchanged between printers from different brands.
- SLA printing costs are comparatively high (e.g. machine, materials, lab environment).

Stereolithography (SLA) – Conclusion

- Although stereolithography is the first process developed for rapid prototyping, and the oldest among the main 3D printing methods, it still remains an attractive solution for creating prototypes with high accuracy and durability.
- Many industries and hobbyists use this process to build prototypes as well as final products, and the technology continues to become more affordable and accessible.

An example of a fractal tree, a complex geometry printable using stereolithography.

Stereolithography (SLA) - Alternative Process: Digital Light Processing

- As we mentioned before, one descendant of SLA is digital light processing (DLP). Unlike SLA, DLP uses a digital projector screen to flash a single image of each layer across the entire platform.
- As the projector is a digital screen, each layer will be composed of square pixels.
- Thus, the resolution of a DLP printer corresponds to pixel size, whereas with SLA, it is the laser spot size.

Digital Light Processing (DLP) 3D Printing

- DLP stands for digital light processing, and is a type of vat polymerization. Vat polymerization 3D printing technologies make use of a (liquid) photopolymer resin which is able to cure (solidify) under a light source.

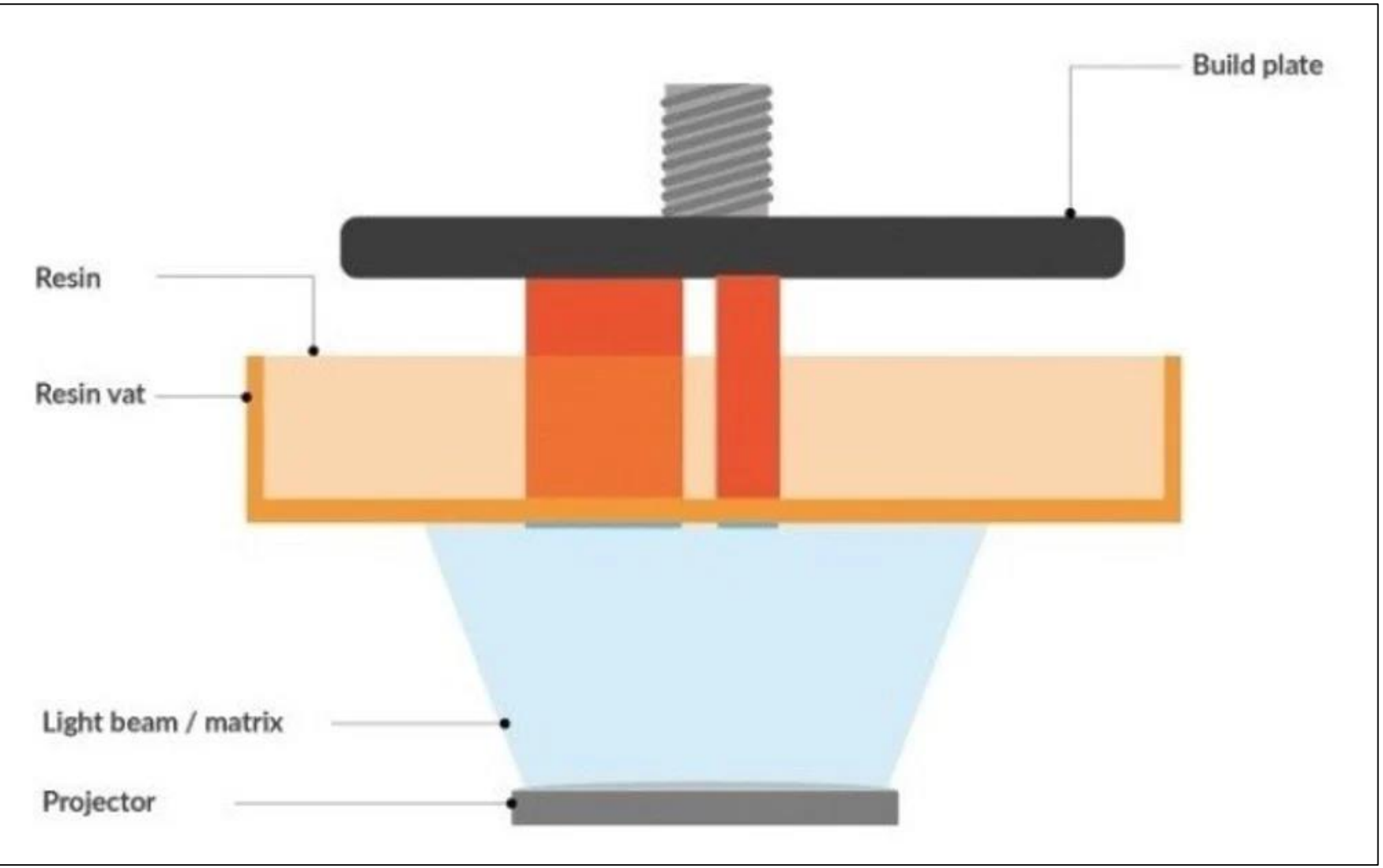
- In the world of vat polymerization, there are two main technologies: SLA and DLP. Naturally, both use resin and a light source to produce parts, the main difference being the type of light source which is used to cure the resin.

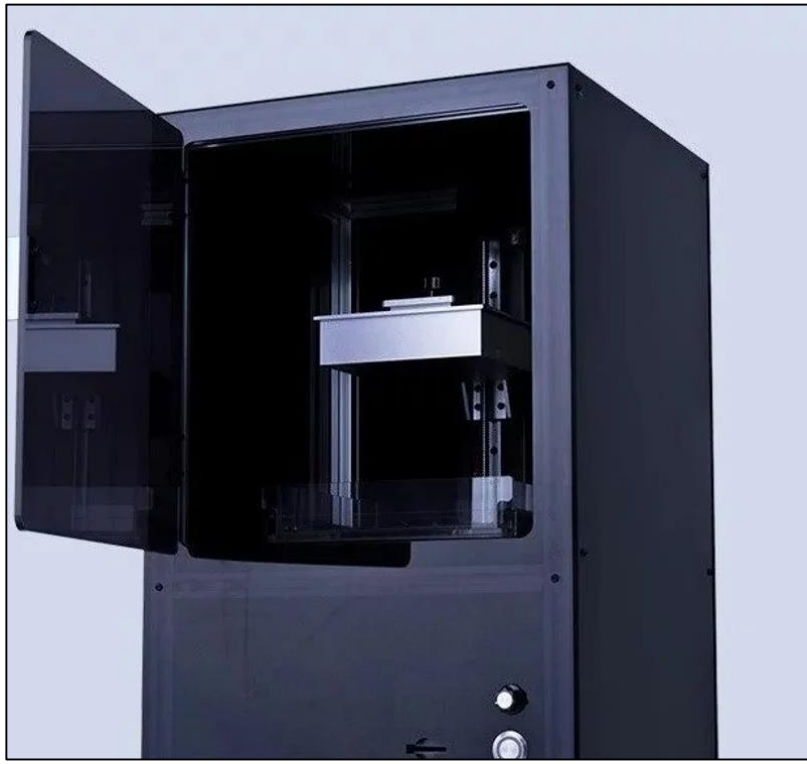
- In order to understand DLP properly, it first makes sense to describe its predecessor, SLA. 3D printers that employ SLA, or stereolithography, use a build platform, an elevator that moves the platform upwards, a tank filled with resin, a light source and galvanometers.

- SLA 3D printers start to work by lowering the build platform into the resin-filled tank with only one layer of height left between the bottom of the tank and the build platform. Then the galvanometers take over.

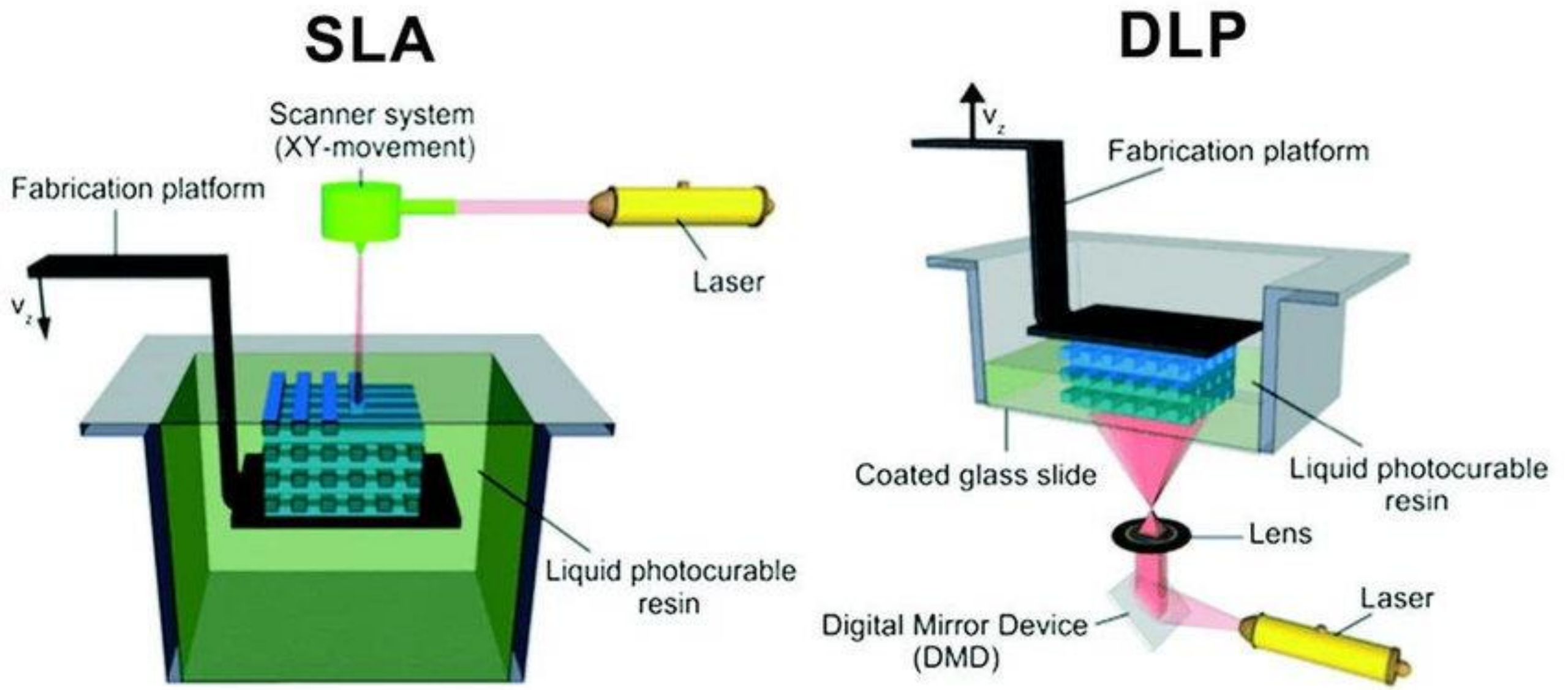
- Galvanometers are mirror-like components used for navigating the laser beam of an SLA printer to the bottom of the tank. With the help of G-code, galvanometers navigate the laser beam in a path which represents one layer of a certain part. The laser then cures the resin making a solid layer of a part. When one layer is complete, the build platform moves up by one layer in height and the process is repeated until the part is complete.

DLP 3D Printing – How does it work?





DLP 3D Printing – How does it work?



DLP 3D Printing – How does it work?

- DLP is a “sister technology” to SLA as the only big difference is the light source used to cure the resin. As we just mentioned, SLA printers use lasers combined with galvanometers to cure resin.

- With a DLP 3D printer, the light source is a specially developed digital light projector screen. Thanks to this screen, DLP is generally considered to be faster than SLA.

- With SLA, the laser has to individually cure the resin in a “point to point” technique. On the other hand, a DLP projector screen flashes an image of a layer all at once. Thus all points of a layer can be cured simultaneously. In this way, the print speed is increased in comparison to SLA since it takes less time to cure a single layer.

- Since the DLP is a digital technology, the 2D image that is projected is composed of pixels. When translated into three dimensions, they become voxels.

- The light source of a DLP 3D printer itself, an LED screen, means nothing without a digital micromirror device (DMD), the “heart” of every DLP chipset. A DMD contains hundreds of thousands or even millions of small micromirrors that direct the light and create the pattern of a layer onto the bottom of the resin tank.

- The resolution of a printed part using a DLP 3D printer usually corresponds to the number of micromirrors inside a DMD device.

FDM 3D Printing

- Fused deposition modeling, or FDM 3D Printing, is a method of additive manufacturing where layers of materials are fused together in a pattern to create an object.
- The material is usually melted just past its glass transition temperature, and then extruded in a pattern next to or on top of previous extrusions, creating an object layer by layer.

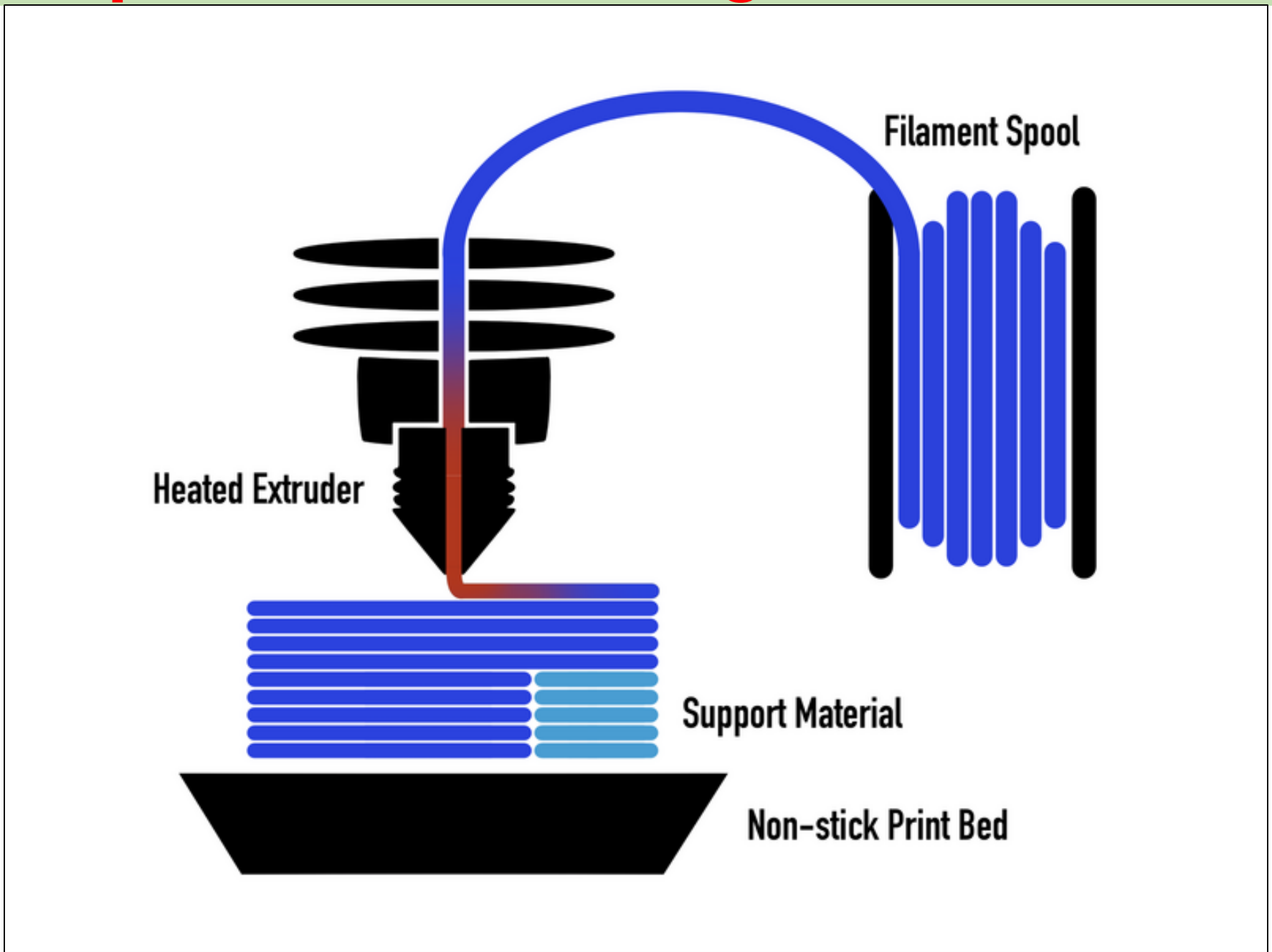
- In layman's terms, a typical FDM 3D printer takes a plastic filament and squeezes it through a hot end, melting it and then depositing it in layers on the print bed. These layers are fused together, building up throughout the print, and eventually they will form the finished part.

- FDM is the same as fused filament fabrication (FFF), but the term "fused deposition modeling" and the abbreviated "FDM" were trademarked by Stratasys in 1991, creating the need for a second name.

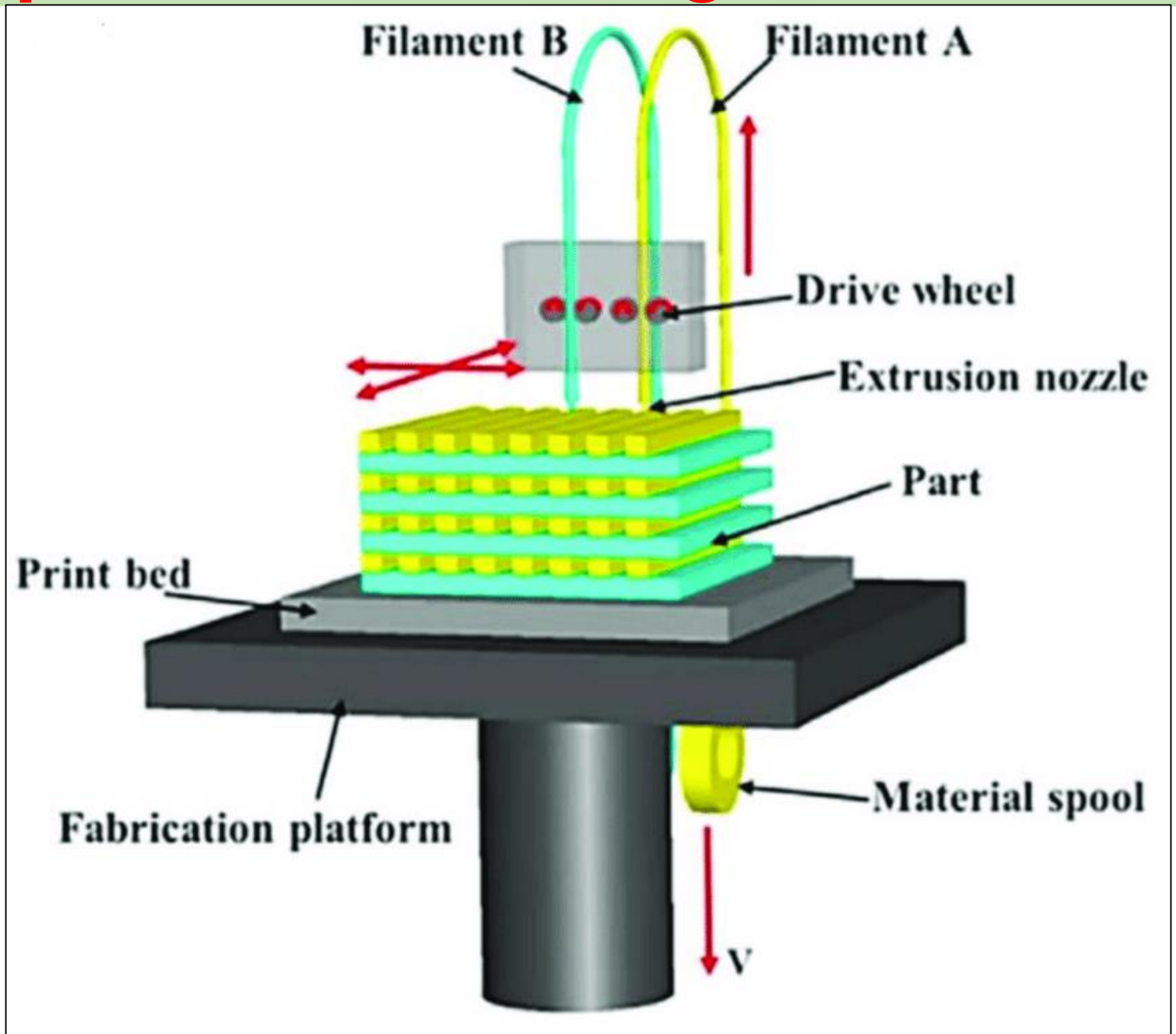
- Many types of materials can be used with FDM techniques, including the most common thermoplastics, chocolate, pastes, and even "exotic" materials like metal- or wood-infused thermoplastic.

- Widely accepted as the simplest way to achieve 3D printing, FDM is cheap and fairly efficient.
- FDM 3D printers dominate the 3D printing market, almost drowning out more expensive methods.

Fused deposition modeling, or FDM 3D Printing



Fused deposition modeling, or FDM 3D Printing



Fused Deposition Modeling (FDM)

- FDM is an assembly-based technology of 3D model fabrication.
- In FDM, a support material is first deposited onto an established base then the main building material is allowed to deposit over the building material and solidify to take the final 3D shape.
- The moveable base is then lowered to allow more layers to build on top of each other in a similar manner.

- Heat applied to provide a semi-molten polymer along with inconsistent pore openings limit its application to thermoplastic polymers. FDM, however, can produce scaffolds with a wide range of porosities and pore sizes.

- FDM can be combined with other techniques.
- As an example of this, FDM was used to fabricate PCL/PLA scaffolds that exhibited a strengthened mechanical profile. Both supercritical CO2 and breath figures mechanisms were employed to enhance the porosity of the FDM-fabricated scaffolds.

FDM 3D Printing – Pros and Cons

- FDM offers a number of advantages over other 3D printing methods, but it also has some downsides.
- **Pros**
- One of the biggest advantages of FDM 3D printing is **scalability**: It can be easily scaled to any size. This is because the only constraint in the size of a build area is the movement of each gantry – make the gantry rails longer and the build area can be made larger. Of course, there are a few minor issues, and at a certain point the cost is no longer offset by the benefits, but no other printer design is capable of being scaled as easily with as few issues as FDM.
- One of the more obvious benefits of having an easily-scalable design is the **cost-to-size ratio**. FDM printers are continually being made bigger and less expensive, due to low part costs and the simple designs involved. Other styles of printer cost many times more per unit area of build volume, simply because they are difficult to scale up and the key components are still quite expensive.
- Another advantage is **material flexibility**. On any FDM printer, a wide variety of thermoplastic materials and exotic filaments can be printed with relatively few upgrades and modifications, something that cannot be said of other styles where a material must be a resin or fine powder.
- **Cons**
- One of the most often referenced downsides of FDM 3D printing is **part quality or detail**. Because the material must be extruded in layers, and has a certain thickness predefined by the nozzle, high detail prints are hard to achieve and often require lots of post-processing to acquire a professional, finished look.
- Another downside of the layers in FDM printing is that they **create and inherent weak point in the print where each layer is joined**, making prints less sturdy and unsuitable for certain applications.

Selective Laser Sintering (SLS)

- Selective laser sintering was one of the first additive manufacturing techniques, developed in the mid-1980s by Dr. Carl Deckard and Dr. Joe Beaman at the University of Texas at Austin.
- Their method has since been adapted to work with a range of materials, including plastics, metals, glass, ceramics, and various composite material powders.
- Today, these technologies are collectively categorized as powder bed fusion—additive manufacturing processes by which thermal energy selectively fuses regions of a powder bed.

- The two most common powder bed fusion systems today are plastic-based, commonly referred to as SLS, and metal-based, known as direct metal laser sintering (DMLS) or selective laser melting (SLM). Until recently, both plastic and metal powder bed fusion systems have been prohibitively expensive and complex, limiting their use to small quantities of high value or custom parts, such as aerospace components or medical devices.

- Innovation in the field has surged recently, and plastic-based SLS is now poised to follow other 3D printing technologies like stereolithography (SLA) and fused deposition modeling (FDM) to gain widespread adoption with accessible, compact systems.

Sintering is a heat treatment process where loose material is subjected to high temperature and pressure in order to compact it into a solid piece.

Selective Laser Sintering (SLS)

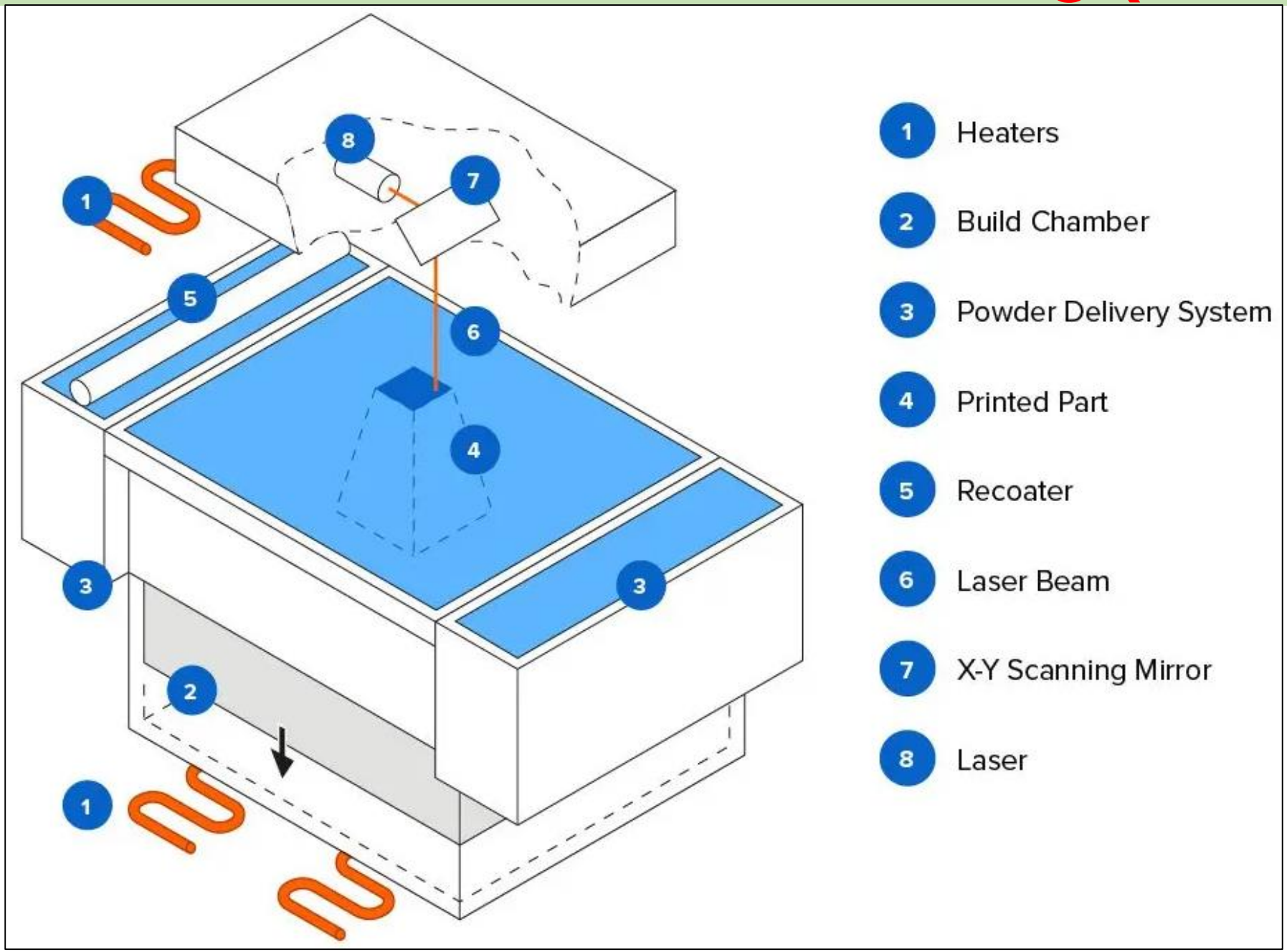
- SLS is the most popular RP laser-based technology.
- **It uses powerful laser beams to fuse and bind powdered particles together in a layer-to-layer manner based on a computer software-assembled 3D model.**
- **Similar to SLA, a 3D multilayer porous structure is obtained through the deposition of multilayers using a moveable platform. The structure shows a low degree of compactness among layers deposited in the sintering procedure, which induces the required porosity.**

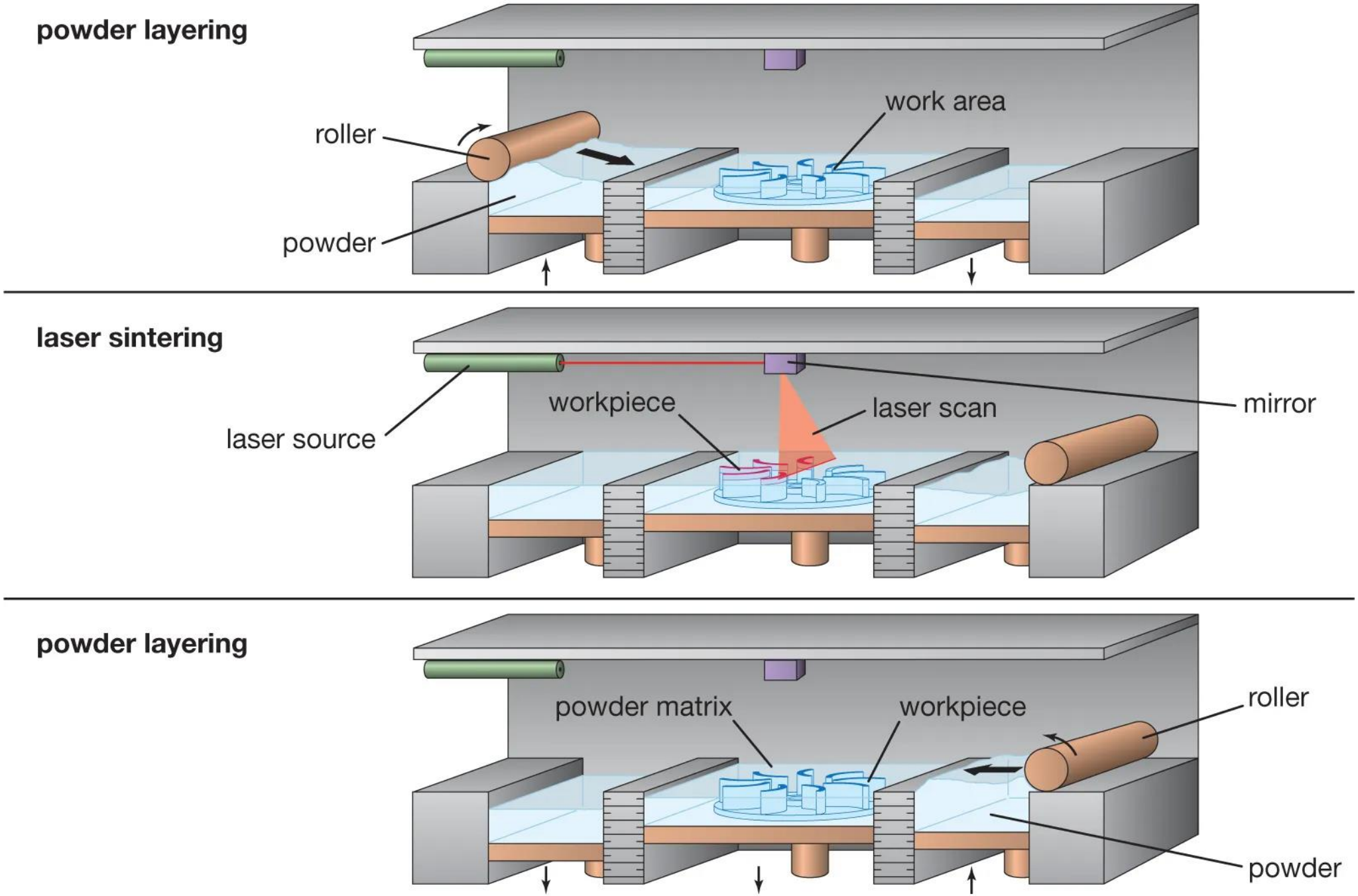
- SLS produces scaffolds of high porosity and pore interconnectivity in shortened processing time.

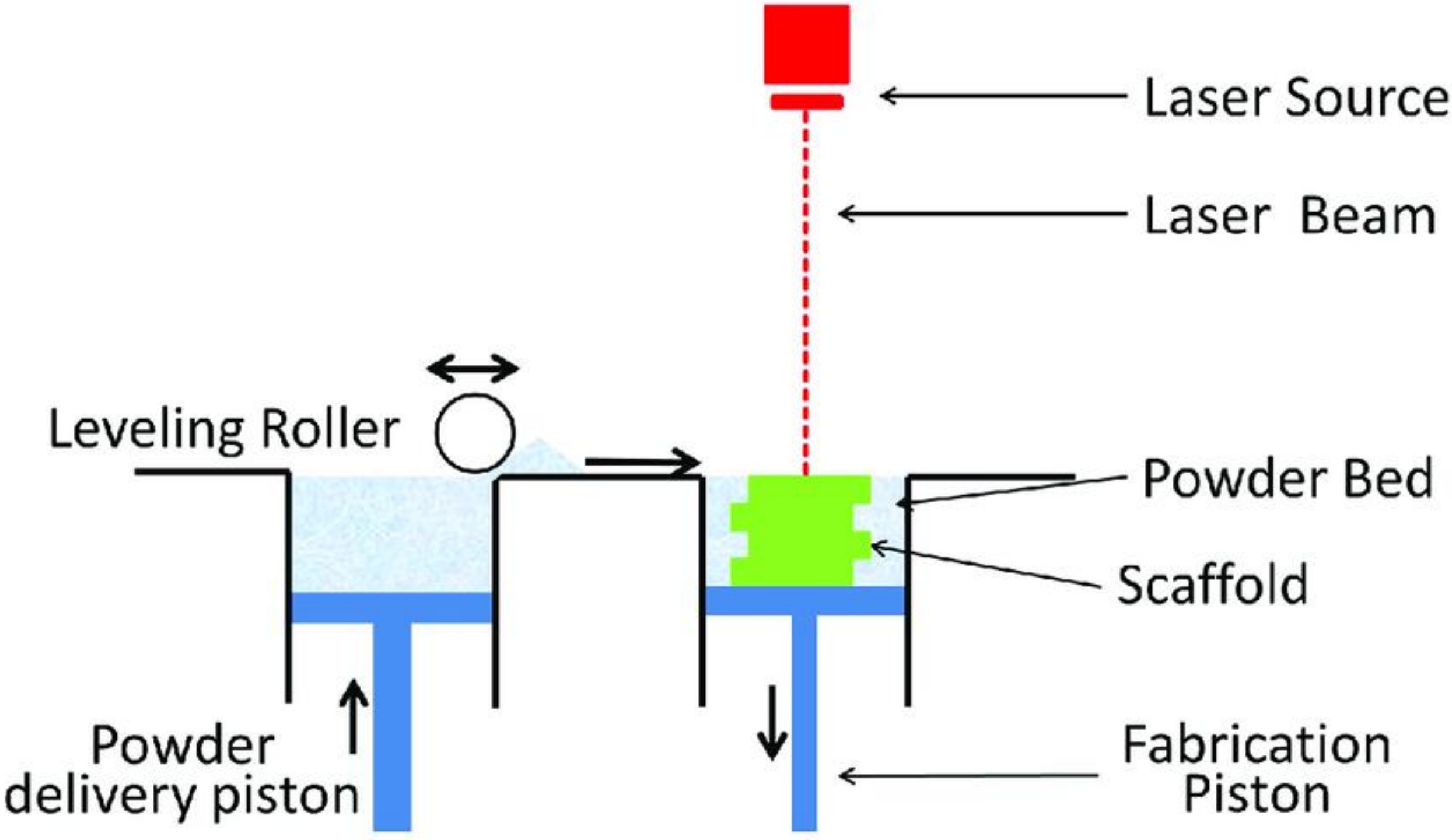
- However, it is difficult to remove the uncured powder, and the method struggles to create small details such as sharp corners and boundaries.

- SLS technique was used to prepare iron (III) oxide (Fe_2O_3)-doped DP-Bioglass for alveolar bone regeneration post dental implantation. The bioglass proved noncytotoxic following WST-1, Live/Dead, and JC-1 stains. As it slowly degraded, it caused the subsequent release of calcium, phosphate, iron, and silica ions, all of which promoted alveolar bone mineralization as seen by xylenol orange staining. Even more, the released ions enhanced osteogenesis following the induction of genes responsible for ALP, collagen type-I, and Runx2.

Selective Laser Sintering (SLS)






Laser Source
Laser Beam
Leveling Roller
Powder Bed
Scaffold
Powder
delivery piston
Fabrication
Piston

Selective Laser Sintering (SLS)

https://www.youtube.com/watch?v=XyFSoIk5OW8

Selective Laser Sintering (SLS)

- **Printing:**
- The powder is dispersed in a thin layer on top of a platform inside of the build chamber. The printer preheats the powder to a temperature somewhat below the melting point of the raw material, which makes it easier for the laser to raise the temperature of specific regions of the powder bed as it traces the model to solidify a part. The laser scans a cross-section of the 3D model, heating the powder to just below or right at the melting point of the material. This fuses the particles together mechanically to create one solid part. The unfused powder supports the part during printing and eliminates the need for dedicated support structures. The platform then lowers by one layer into the build chamber, typically between 50 to 200 microns, and the process repeats for each layer until parts are complete.

- **Cooling:**
- After printing, the build chamber needs to slightly cool down inside the print enclosure and then outside the printer to ensure optimal mechanical properties and avoid warping in parts.

- **Post-processing:**
- The finished parts need to be removed from the build chamber, separated, and cleaned of excess powder. The powder can be recycled and the printed parts can be further post-processed by media blasting or media tumbling.

SLS - What is SLS and How Does It Work?

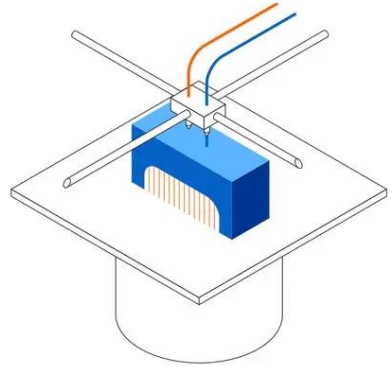
- Selective laser sintering (SLS) is an additive manufacturing (AM) technology that uses a high power laser to sinter small particles of polymer powder into a solid structure based on a 3D model.
- Selective laser sintering (SLS) 3D printers make use of a laser and a thermoplastic polymer powder to build parts. Because of the high power laser, it's generally considered more complicated than both FDM and SLA.
- SLS 3D printers consist of a powder bin, a build platform, a powder re-coater, a laser (either CO2, diode, or fiber), a set of galvanometers, a set of heaters, and a powder feeder.
- Generally speaking, the printing process begins with filling the powder bin with a certain amount of polymer powder. The bin is then placed into the machine, where the heating stage begins. Before printing, heaters are used to get the powder to a temperature just below its melting point.
- The actual printing starts with the re-coater depositing a single layer of powder onto the build platform. That's where the CO2 laser comes into play. The laser's role is to selectively induce fusion between particles to form a solid at specific locations.
- Galvanometers are used to navigate the laser beam to a specific point on the build platform. If you don't know what galvanometers are, think of them as tiny mirrors.

- The laser moves along a "point-to-point" pattern, solidifying the whole cross-sectional area of a layer. After the layer is complete, the re-coater deposits a new layer of powder and the build platform moves down one layer in height. The process is then repeated until the part is complete.

SLS – Pros and Cons

- The biggest advantage of SLS is that there's no need for additional support material, regardless of the part's geometry.
- As parts are built inside the powder bin, the non-sintered powder acts as a support material to the printed part. After the part has been printed, it's left with no markings of the support material on its surface. The powder need only be brushed away.
- Apart from this unique advantage, there are a few more. Because parts are built by a laser, which solidifies a cross-sectional area of each part, multiple parts can easily be printed at once. This makes SLS ideal for small manufacturing runs. The goal with SLS printers is to fill the build area with as many prints as possible so as to reduce the non-sintered waste powder.
- Notably, 50% of the non-sintered powder can be recycled.
- Unlike FDM, parts printed with an SLS printer cannot be used right after the print process is complete. That's because parts must cool down, which can take a long time.
- The most significant disadvantage of SLS is that it requires high-end technology and a lot of power. As such, it's not quite ready to hit the consumer market.

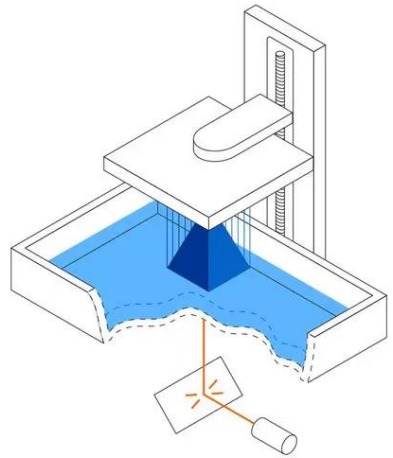
- **SLS 3D printers are ideal for small manufacturing runs as the goal of every SLS 3D print is to produce as many parts at once. This both improves the speed of manufacturing and reduces the waste of non-sintered and non-recyclable powder.**



FDM
Fused Deposition Modeling

- Melts and extrudes thermoplastic filament
- Lowest price of entry and materials
- Lowest resolution and accuracy

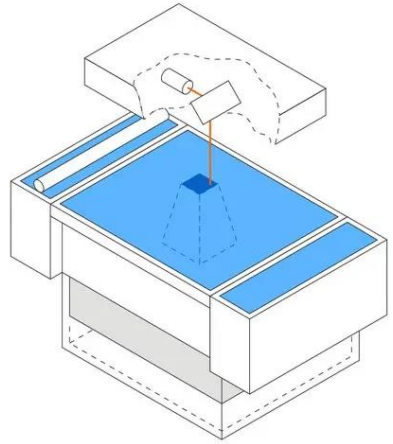
BEST FOR:
Basic proof-of-concept models and simple prototyping



SLA
Stereolithography

- Laser cures photopolymer resin
- Highly versatile material selection
- Highest resolution and accuracy, fine details

BEST FOR:
Functional prototyping, patterns, molds and tooling



SLS
Selective Laser Sintering

- Laser fuses polymer powder
- Low cost per part, high productivity, and no support structures
- Excellent mechanical properties resembling injection-molded parts

BEST FOR:
Functional prototyping and end-use production

SLA vs. DLP

SLA vs. DLP

- Up to this point, the benefit of a DLP 3D printer over an SLA machine: the increased speed.

- But, DLP also has its limitations.

- Since a DLP 3D printer uses a digital projector screen, the resolution of your print directly corresponds to the resolution of your projector. In other words, a cheaper projector will likely mean reduced resolution. This is in contrast to SLA, where even cheaper devices are likely to have decent resolution (i.e. a narrow beam width).

- Another limitation of DLP printers is the boxy surface finish. Since voxels are rectangular, curved sections of a print tend not have a very smooth finish when compared to SLA. The good thing is that the problem with voxels and curves can be solved by sanding the part after printing.

- Both DLP and SLA printers use resins, so the cost of printing should be similar. However, keep in mind that cost greatly depends on the manufacturer of the resin or even the printer.

SLA vs. FDM

SLA vs. FDM

- In FDM, filament is fed through a hot extruder and deposited layer-by-layer. The materials used are typically thermoplastics, but they can be mixed with other elements, including wood, metal, and carbon fiber. This is one advantage over SLA, which has only a limited choice of materials.

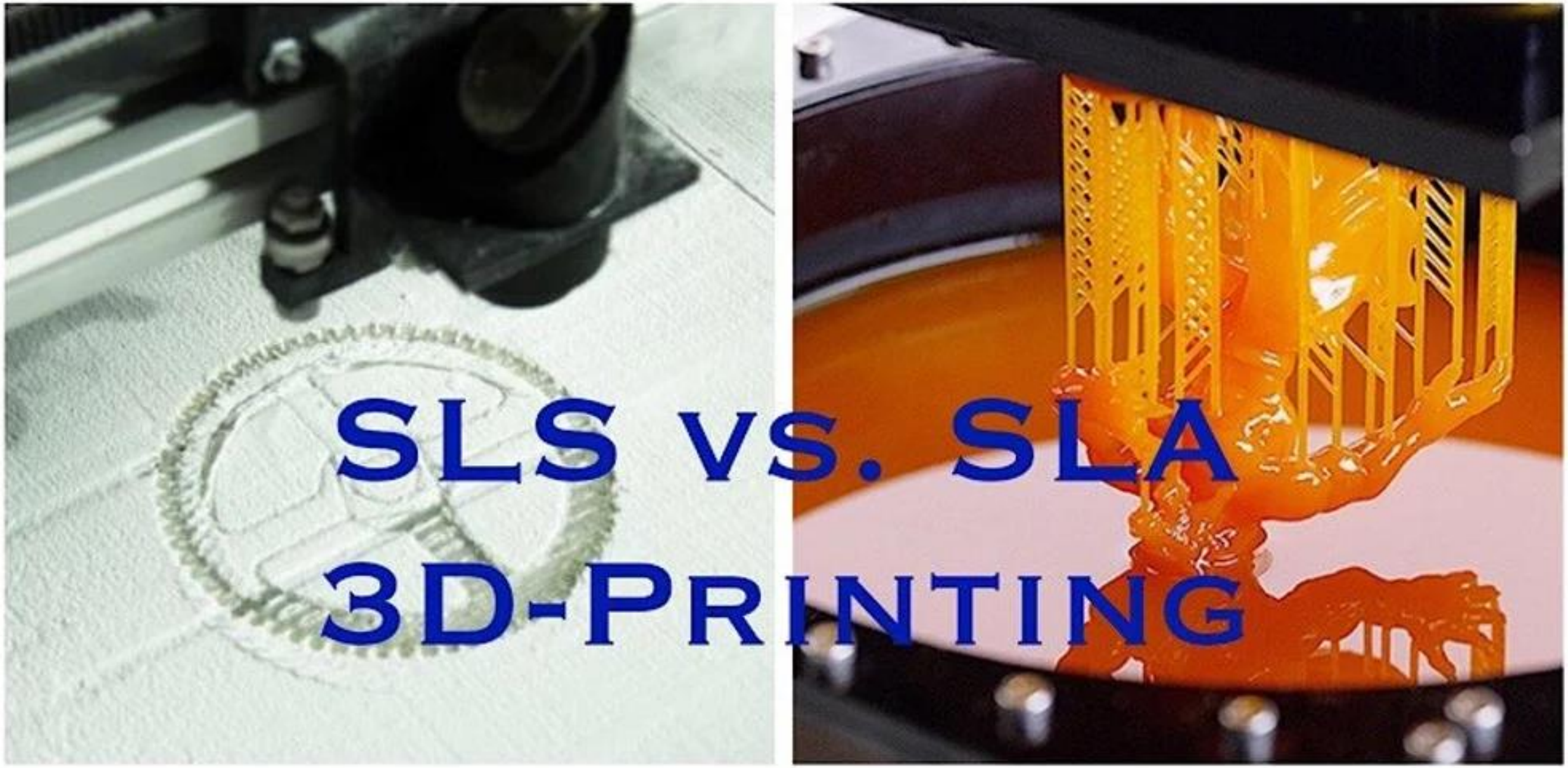
- Whereas in FDM resolution refers to the precision of the motors, in SLA it depends on the tightness of the laser beam. This is why SLA is capable of producing objects of higher detail and accuracy.

- An object printed using an FDM 3D printer is post-processed by removing supports (if present) and smoothening surfaces. In SLA, prints are submerged in isopropyl alcohol to remove excess resin before being subjected to passive UV light for extra strengthening. Nevertheless, the final result is in general not as strong as a product of FDM.

- The cost of materials is decisively lower for FDM, since the printers are more affordable, and plastic reels are cheaper than resin.

- In a nutshell, if high precision and a smooth finish are priorities, SLA will be your best bet. If cost and (to a lesser extent) durability play a role, use an FDM printer.

SLA vs. SLS



SLS vs. SLA

SLA vs. SLS

- Selective laser sintering (SLS) involves an entirely different approach, although it also involves the use of a laser.

- Although it also uses a laser, it's much more powerful. That's because, instead of curing a substance, the beam heats a powder to the point of fusing its particles together. Often grouped with SLS are direct metal laser sintering (DMLS) and selective laser melting (SLM), which are specifically adapted to metals. Normal SLS works with polymers like nylon.

- Compared to objects made with other technologies, SLS prints are especially strong and durable. Also, because supports aren't necessary in SLS, prints can be of complex geometries. Detailing can be fairly high with SLS, it generally can't compare to the precision of SLA.

- Because of their highly powered lasers, SLS machines incorporate more advanced technology, including special shielding against harmful UV radiation. This results in printers that are more expensive, with few desktop or benchtop options available.

- Furthermore, SLS powders are more expensive than liquid photopolymers.

- In a nutshell, if high mechanical strength and complex shapes are your priority, and the cost is of minor importance, use an SLS printer. Otherwise, SLA is probably your best bet.

Three-Dimensional Printing (3DP) and Bioprinting

- 3DP is another laser-based technology that uses a CAD model to obtain a 3D structure.

- A 3D ink-jet printer is used to distribute a layer of powder on the moveable platform. Following, droplets of a liquid binder are jetted over the preformed layer to bind the particles together. Different layers are deposited above each other in a similar manner.

- Finally, unbound powders are removed, and a 3D model is obtained. 3DP is fast emerging owing to its cost-effectiveness and rapid conversion rates of CAD files into 3D constructs. The technique is widely versatile and can be used in the production of constructs with fine details; however, FDM uses a binder liquid that requires its complete removal post processing, which is a tedious and often incomplete process.

- Porous titanium alloy-based scaffold, Ti-6Al-4V, was fabricated using 3DP and tested in vivo for possible tendon fixation following prosthetic implantation. Micro-CT and hard tissue staining visualized the increased fibroblasts adhesion to and growth into the 3D-printed scaffolds of 527.15 µm pore size.

Three-Dimensional Printing (3DP) and Bioprinting

- 3D Bioprinting is an extension of 3D Printing used to produce pre-tissues, tissues, or complete organs based on the same technique implemented in 3DP.

- Different approaches are used in bioprinting, including autonomous self-assembly and biomimicry for full organs and small building blocks for pre-tissues and tissues. Bioprinting can either be used to produce cellular or acellular constructs, with acellular ones being the easier approach. The omission of cells in acellular constructs means fewer restrictions during the manufacturing process.

- Bioprinting, by fabricating 3D organs, indeed could be the solution to the limited supply of organ transplants.

- Advanced techniques are not in the early stages anymore to be used in TE as sole replacements to the conventional methods. Even more, the benefits added, particularly overcoming cell supply shortcomings and the much shorter time needed to generate a full tissue of an organ, are worthy of research and development.

Three-Dimensional Printing (3DP) and Bioprinting

- 3D bioprinting is an additive manufacturing process where organic and biological materials such as living cells and nutrients are combined to create artificial structures that imitate natural human tissues.
- 3D boprinting is an additive manufacturing process where biomaterials such as cells and growth factors are combined to create tissue-like structures that imitate natural tissues.
- In other words, bioprinting is a type of 3D printing that can potentially produce anything from bone tissue and blood vessels to living tissues for various medical applications, including tissue engineering and drug testing and development.
- Perhaps the most significant driver of 3D bioprinting is regenerative medicine. According to Chris Mason and Peter Dunnill, this involves replacing or regenerating “human cells, tissue, or organs, to restore or establish normal function.” Here, bioprinting can have a central role, especially considering the high demand for organ and tissue transplants worldwide.

3D bioprinted "living" ear developed in 2016

What Is 3D Bioprinting?

- Bioprinting is an additive manufacturing process where biomaterials such as cells and growth factors are combined to create tissue-like structures that imitate natural tissues.

- The technology uses a material known as bioink to create these structures in a layer-by-layer manner. The technique is widely applicable to the fields of medicine and bioengineering. Recently, the technology has even made advancements in the production of cartilage tissue for use in reconstruction and regeneration.

- In essence, bioprinting works in a similar way to conventional 3D printing. A digital model becomes a physical 3D object layer-by-layer. In this instance, however, a living cell suspension is utilized instead of a thermoplastic or a resin.

- For this reason, in order to optimize cell viability and achieve a printing resolution adequate for a correct cell-matrix structure, it’s necessary to maintain sterile printing conditions. This ensures accuracy in complex tissues, requisite cell-to-cell distances, and correct output.

Three-Dimensional Printing (3DP) and Bioprinting

- The products obtained from bioprinting technologies can mimic both the biological and functional properties of our bodies' natural-occurring structures and tissues.

- This can potentially lead to different kinds of applications, but today there's only one feasible use for bioprinting: pharmaceutical drug testing and research.

- While the ultimate goal of 3D bioprinting is the production of artificial organs for transplantation, the complexity involved in making them function as real organs is huge. However, scientists today can successfully create biological structures and tissues that imitate natural ones.

- So, instead of bioprinting fully functional kidneys, researchers can already create structures that chemically behave like kidney tissue. While far from the original goal, these structures can be used to test new drugs without having to rely on real-life patients that could suffer from unexpected side effects.

- Besides the ethical part of it, drug development with bioprinted materials can make pre-clinical trials of new drugs much more cost-effective, helping them to be validated and reach the market sooner, while also potentially reducing the need for animal testing.

Three-Dimensional Printing (3DP) and Bioprinting

- Yet, 3D bioprinting started as a regenerative medicine tool. The production of artificial organs for transplantation would solve the issues of high demand and low availability, as well as post-surgical complications associated with organ rejection given that the fabricated organs would be developed using the patient's own organic material.

- While organ replacement is the ultimate objective, in the meantime, tissue repair has been showing very promising results. Instead of creating entire functional organs, the small tissue patches can be potentially used to regenerate and treat organs like the liver and heart. Bone and skin grafting can also benefit from the technology, including surgery for reconstructive and aesthetic purposes.

- Both these applications associated with regenerative medicine are still in development, with only a handful of successful cases in research labs and animals such as the University of Toronto's project of "printing" skin on burn injuries.

3D Bioprinting – How does it work?

- In essence, 3D bioprinting works similarly to conventional 3D printing: digital models are transformed into physical three-dimensional objects via layer-by-layer fabrication techniques.
- Several bioprinting methods are available, including those based on extrusion, where materials are discharged from a needle to create layers, and laser-assisted technologies, where the laser acts as a heat source to help deposit materials onto the substrate, in a process similar to selective laser melting (SLM).

- Regardless of the bioprinting method, instead of inorganic raw materials such as filament, resin, and metal, here, the feedstock material is composed of biological agents and living cells.
- These materials are known as bioinks, which are mainly composed of living matter like cells within a carrier material – like collagen, gelatin, hyaluronan, silk, alginate, or nanocellulose – that act as a molecular scaffold for the structure to grow and nutrients to provide support.

- In general terms, the bioprinting process as a whole involves several steps which can be summarized in three key stages: the preparation, which consists of creating the digital model (analogous to the 3D modeling stages of conventional 3D printing); the layer-by-layer construction process itself; and the post-bioprinting stages that involve mechanical and chemical stimulation to stabilize structures and mature biological material.

Key Steps of Bioprinting

- Despite the various 3D bioprinting types and methods, a typical process follows roughly the same standard series of steps.
- **Preparation**
- **3D imaging:**
- Similarly to what 3D scanning is to conventional 3D printing, normal computed tomography (CT) and magnetic resonance imaging (MRI) scans are utilized to get as much information as possible about biological structures and their surroundings.

- **3D modeling:**
- The actual three-dimensional model of the structure or tissue is generated by special software. The model is developed on a microscale, already considering the layer-by-layer fabrication method and the carrier materials.

- **Bioink preparation:**
- The bioink type and preparation depend on the printing method of the particular bioprinter. For instance, in extrusion-based bioprinting methods, the bioink should be a highly viscous fluid to keep a relative structure after extrusion (the action of thrusting or forcing something out), whereas for other methods such as inkjet or those involving microfluidity, the feedstock materials must be in a more liquid state in to flow properly.

Key Steps of Bioprinting

- **Construction (or 3D printing)**
- **Printing:**
- Regardless of the method, the 3D bioprinting process involves depositing the material layer-by-layer. The printing resolution can reach incredible single-cell deposition in some bioprinting methods, like Fluicell's microfluidic flow printing.

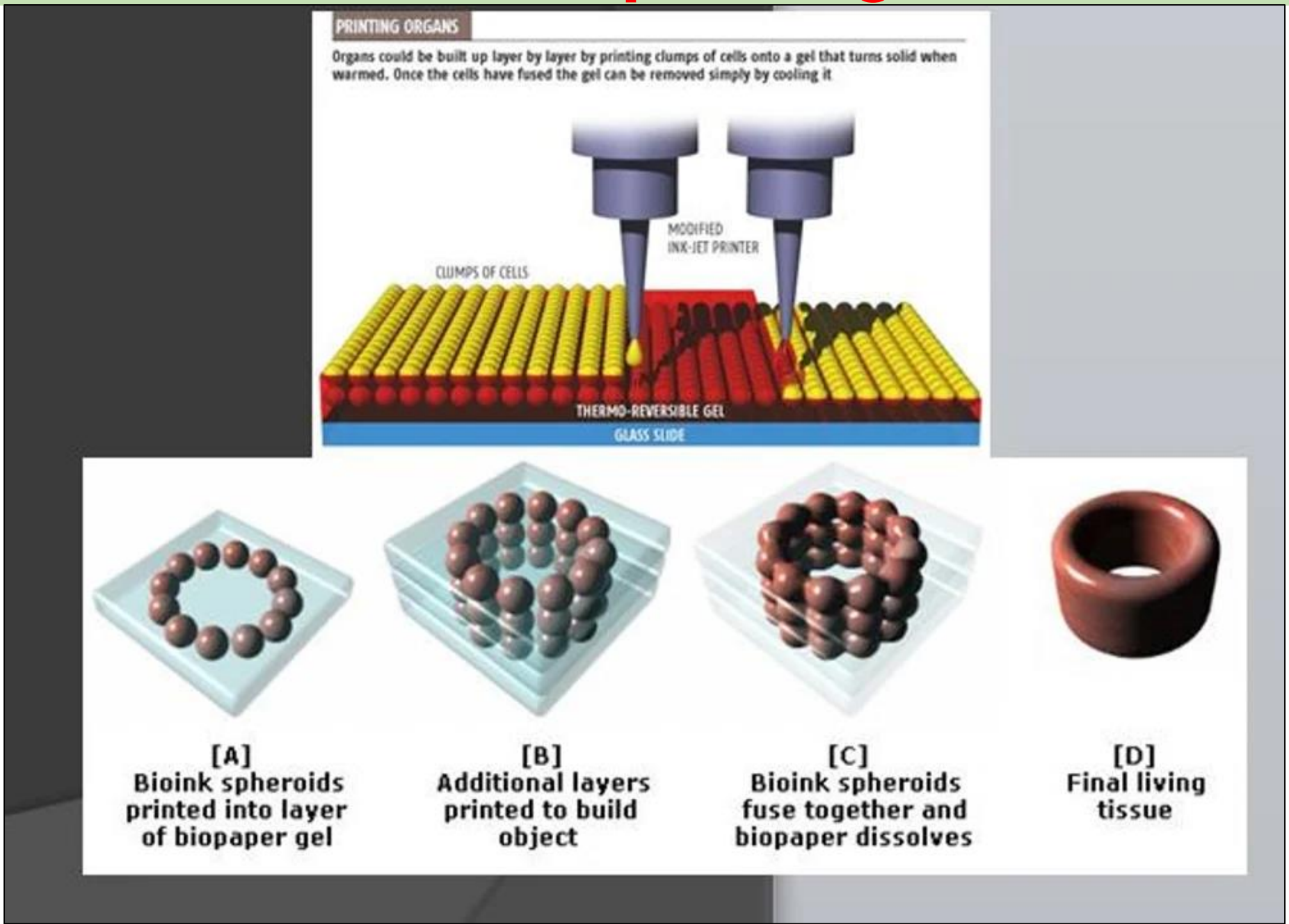
- **Post-Bioprinting**
- **Crosslinking:**
- Once printed, the materials are still in a relatively sluggish state, so an extra step is necessary to properly solidify and blend them together. This is known as crosslinking, and it's an essential step to ensure the mechanical and chemical properties of the printed structure. Crosslinking can use different environmental controls like UV light, temperature, and chemicals, among others.
- **Maturation:**
- Finally, the bioprinted and crosslinked structures need to grow – biologically. This means that the printed living cells will reproduce, and tissue will grow following the underlying printed structures. This step is also called incubation and is done inside bioreactors that create a favorable environment for reproduction and tissue growth.

How Does Bioprinting Work?

- Several bioprinting methods exist, based on either extrusion, inkjet, acoustic, or laser technologies. Despite the various types, a typical bioprinting process has a more-or-less standard series of steps:

- **3D Imaging:** To get the exact dimensions of the tissue, a standard CT or MRI scan is used. 3D imaging should provide a perfect fit of the tissue with little or no adjustment required on the part of the surgeon.
- **3D Modeling:** A blueprint is generated using AutoCAD software. The blueprint also includes layer-by-layer instruction in high detail. Fine adjustments may be made at this stage to avoid the transfer of defects.
- **Bioink Preparation:** Bioink is a combination of living cells and a compatible base, like collagen, gelatin, hyaluronan, silk, alginate or nanocellulose. The latter provides cells with scaffolding to grow on and nutriment to survive on. The complete substance is based on the patient and is function-specific.
- **Printing:** The 3D printing process involves depositing the bioink layer-by-layer, where each layer has a thickness of 0.5 mm or less. The delivery of smaller or larger deposits highly depends on the number of nozzles and the kind of tissue being printed. The mixture comes out of the nozzle as a highly viscous fluid.
- **Solidification:** As deposition takes place, the layer starts as a viscous liquid and solidifies to hold its shape. This happens as more layers are continuously deposited. The process of blending and solidification is known as crosslinking and may be aided by UV light, specific chemicals, or heat (also typically delivered via a UV light source).

How Does Bioprinting Work?



What Is 3D Bioprinting?

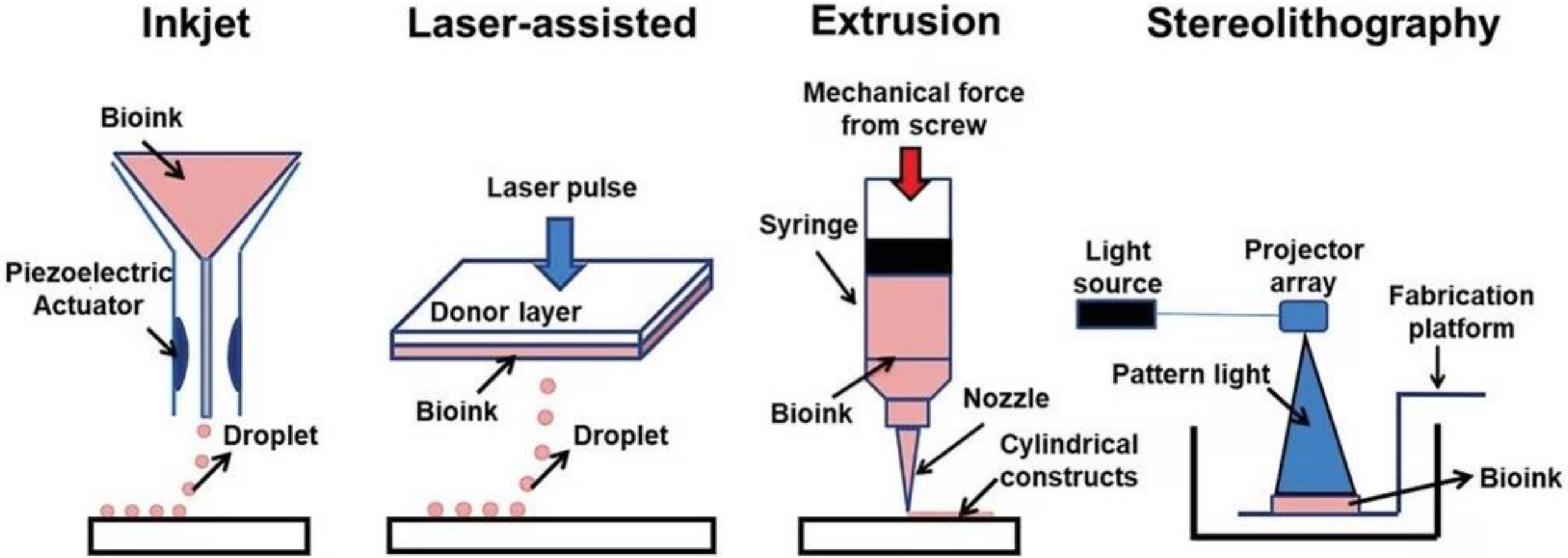
- The process principally involves preparation, printing, maturation, and application. This can be summarized in the three key steps:

- Pre-bioprinting involves creating the digital model that the printer will produce. The technologies used are computed tomography (CT) and magnetic resonance imaging (MRI) scans.

- Bioprinting is the actual printing process, where bioink is placed in a printer cartridge and deposition takes place based on the digital model.

- Post-bioprinting is the mechanical and chemical stimulation of printed parts so as to create stable structures for the biological material.

3D Bioprinters



The working principle of a piezoelectric actuator is, once the voltage is applied to piezoelectric actuators then they generate a small displacement through a high force capacity, so these are used in many applications like ultra-precise positioning, in high forces handling & generation in stationary or dynamic conditions.

3D Bioprinting

- There are four of the common types of bioinks. These are primarily classified by the method through which they undergo the transition from a liquid to a solid or a gel.
- The first is through ionic crosslinking where the ink is printed directly into a crosslinking solution.
- The second is a bioink which is susceptible to changes in temperatures, it is a liquid at a higher temperature (within the syringe) and becomes a gel once it comes into contact with the platform which is a cooler temperature.
- The third is photosensitive bioink which reacts once they are exposed to UV-light.
- The last common bioink are those which undergo gelation due to the shear-thinning forces that it undergoes whilst printing (shear thinning is the non-Newtonian behavior of fluids whose viscosity decreases under shear strain).

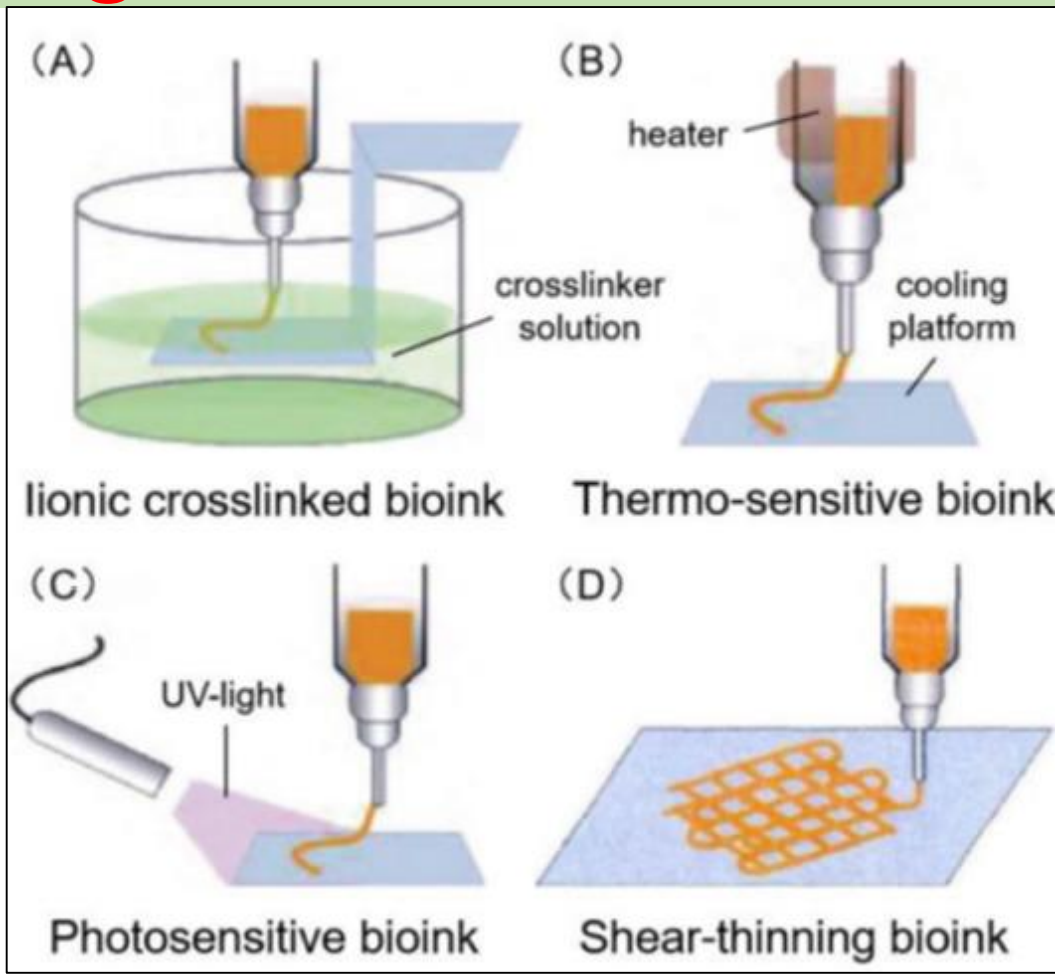


Figure. Illustrate above are the four common types of bioink. These undergo a transition from a liquid to a solid or gel as a response to either (A) an ionic crosslinking agent, (B) a change a temperature, (C) exposure to UV-light or (D) shear-thinning forces.

Three-Dimensional Printing (3DP) and Bioprinting

- 3D bioprinting, which refers to the **use of inks which contain cells**, is a type of 3D printing which is of particular interest in terms of tissue engineering.

- There are a number of different techniques which can be employed in 3D bioprinting formulations.

- The first of which is inkjet-based printing. This technique places small droplets of the bioink onto a substrate, either in a continuous stream or on a “drop-on-demand” basis.

- The second technique is extrusion based, where the ink is loaded into a syringe and forced out of through a nozzle, creating a continuous filament without the creation of droplets. Extrusion based printing requires the ink to be highly viscous whilst still being able to flow out of the syringe without the presence of an elevated temperature.

- The third technique, known as laser-based or orifice-free printing, involves the use of a laser to guide the placement of the ink onto the substrate.

Bioprinting Applications

- **Here are a few of the main application areas of bioprinting:**

- Artificial organs are one of the greatest drivers of the technology due to the high rise of vital organ failure. Availability of 3D printed organs helps to solve organ-related issues faster and quicker, which is important to patients, their families, and healthcare systems.

- Development of tissues for pharmaceutical testing, when 3D printed, is a more cost-effective and ethical option. It also helps in identifying side effects of drugs and allows recommended drugs to be administered to humans with validated safe dosages.

- Cosmetic surgery, particularly plastic surgery and skin grafting, also benefits from the technology. In this particular application, bioprinted skin tissue could be commercialized. Some 3D printed tissues are already being bioprinted for research on therapeutic purposes.

- Bone tissue regeneration as well as prosthetics and dental applications.

3D Bioprinting

- Lee et al., developed a 3D bioprinted system to mimic human skin whereby keratinocytes and fibroblasts are able to represent the epidermis and dermis. The system was comprised of a collagen hydrogel ink which was printed in a layer-by-layer design, alternating with the printing of cells.

- The final structure was made up of eight printed collagen layers. The fibroblast layers were printed after every two layers of collagen (three layers in total) and two layers of keratinocytes were printed on top of the last layer of collagen in order to replicate the cell density of the epidermis.

- The 3D printed system was tested against manually fabricated tissue samples with positive results. The printed system was able to maintain its shape, structure and physical dimension more effectively.

- This research showed that the 3D printing of skin tissues allows for improved control over the cell location as opposed to the traditional manual deposition method and is a feasible method for the creation and reconstruction of skin tissues.

Lee, V.; Singh, G.; Trasatti, J.P.; Bjornsson, C.; Xu, X.; Tran, T.N.; Yoo, S.-S.; Dai, G.; Karande, P. Design and fabrication of human skin by three-dimensional bioprinting. Tiss. Eng. C. Meth. 2013, 20.

Why Is Bioprinting Important?

- The greatest importance of bioprinting lies in the resulting tissue-like structures that mimic the actual micro- and macro-environment of human tissues and organs. This is critical in drug testing and clinical trials, with the potential, for example, to drastically reduce the need for animal trials.

- When living tissues and organs need not come from humans, this budding technology offers other massive opportunities. One example is testing treatment for diseases using artificially affected tissues.

- The process could also eradicate the headaches associated with organ donation and transplantation. Apart from the lack of available organs, the entire process is criticized from a moral and ethical perspective.

- Organ replacement is the main objective, but tissue repair is also possible in the meantime. With bioink, it’s much easier to solve problems on a patient-specific level, promoting simpler operations.

Technique	Advantages	Disadvantages
3D printing (3DP)	• Possibility of using hydrogels and cells	• Low precision • Long-standing process • Poor mechanical properties
Selective laser sintering (SLS)	• Smart process • High precision • No need for support • Construction	• High temperature • Rough surface
Stereolithography (SLA)	• High precision • Smart process • Soft surface	• Risk of high process temperature • Untreated • Material may be cytotoxic • high costs
Fused deposition modeling (FDM)	• Good mechanical properties	• Poor precision • High temperature • Narrow range of parameters • Limits in application to biodegradable polymers

Review

Review of Synthetic and Hybrid Scaffolds in Cartilage Tissue Engineering

Monika Wasyłeczko *, Wioleta Sikorska and Andrzej Chwojnowski

Scaffold fabrication techniques

Table 1 List of techniques used in scaffold fabrication

Techniques	Properties	Pore size/pore density	References
Solvent casting/particulate leaching	• Only the porogens and polymer remain after evaporation • Properties can be tuned accordingly by changing the salt concentrations/size and polymer • Low cost and easy	30–300 µm 75–88%	[195–197]
Thermally induced phase separation	• Pores are formed when the solvent is evaporated Materials/agents (depending on the target tissue) to the primary polymer solution can be easily incorporated • Varying some TIPS processing parameters like polymer concentration (from 2.5 to 15 wt%) and freezing temperature (from 4 to − 60 °C) structural and mechanical properties could be controlled	1–100 µm 95%	[196, 198]
Self assembly	• Material very similar to ECM can be developed • Components are organized into patterns/structures autonomously • Used for nerve and cartilage tissue engineering	5–200 nm	[199]
Freeze drying	• The solution is cast and frozen at − 70 to − 80 °C and dried in a low-pressure chamber • Extraction of material takes place inside the chamber by sublimation and the final drying occurs due to desorption • Porosity can be controlled by maintaining the freeze-drying pressure accordingly	20–200 µm 90%	[197, 199]
Gas foaming	• A foaming agent, e.g., sodium bicarbonate, is used • High pressure is required to initiate nucleation and development of gas and then lyophilized • Liberation of gas forms the pores	40–800 µm 85%	[16, 197]
Electrospinning	• Electrostatic force is used to produce fibres • When a liquid is charged at a high voltage, the surface tension and electrostatic repulsion interact, causing droplets on the spinneret to erupt and stretch • Variables: material used, collector setup, post-processing	10 nm–6 µm (fibre diameter) 80–95%	[6, 197, 200]
Rapid prototyping	• Stereolithography, selective laser sintering, solvent-based extrusion free forming, bioprinting, fused deposition modelling • Various types of 3D structures could be fabricated • Accurate cell–cell interactions in a 3D environment by controlling geometry at the micro and nano cellular levels • Layer by layer arrangement with interconnected pores	25–150 µm	[199, 201]

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Review

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Advancing strategies towards the development of tissue engineering scaffolds: a review

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Scaffold fabrication techniques

Table 1. Classification of scaffold fabrication techniques used in skin tissue engineering.

Fabrication Techniques		Advantages	Disadvantages
Conventional fabrication techniques	Electrospinning	Essential for developing nanofibrous scaffolds, homogenous mixtures made of fibres with high tensile strength [24]	Process depends on many variables, problematic to obtain 3D structures with the required pore size needed for biomedical application [26,27]
	Freeze drying	Used in a variety of purposes, capability of obtaining high temperature, manageable pore size by changing freezing method [24]	High energy consumption, long term timescale, generation of irregular size pores [28]
	Gas foaming	Porosity up to 56.71% [29]	Temperature dependent, product obtained from decreased temperature might have closed pore structure or a solid polymeric skin [30]
	Thermal induced phase separation	Porosity up to 80% [31], can use low temperature to integrate bioactive molecules [24]	Only used for polymers amenable to phase separation [31]
Rapid prototyping (RP)	Bioprinting	Low cost, higher accuracy, and greater shape complexity [24]	Depends on the cells/biomaterials used [32]
	Fused deposition modelling (FDM)	High tensile strength [24]	Has limited application to biodegradable polymers [33]
	Solvent based extrusion free forming (SEF)	Used to make ceramic, metal, and metal/ceramic composite part; used for precise control of scaffold structure at the micron level [24]	Variation in temperature affects extrusion pressure, including nozzle length-to-diameter ratio, and the extrusion velocity [34]
	Stereolithography	High resolution, uniformity in pore connectivity [24]	Requires a massive number of monomers and post-polymerization treatment to improve monomer conversion [35,36]

polymers

MDPI

Review

Synergistic Effect of Biomaterial and Stem Cell for Skin Tissue Engineering in Cutaneous Wound Healing: A Concise Review

Shaima Maliha Riha, Manira Maarof and Mh Busra Fauzi *

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Thank you for your attention