

Determination of Resin Components for continuous Digital Light Processing (cDLP) Additive Manufacture of Resorbable Tissue Engineering Scaffolds

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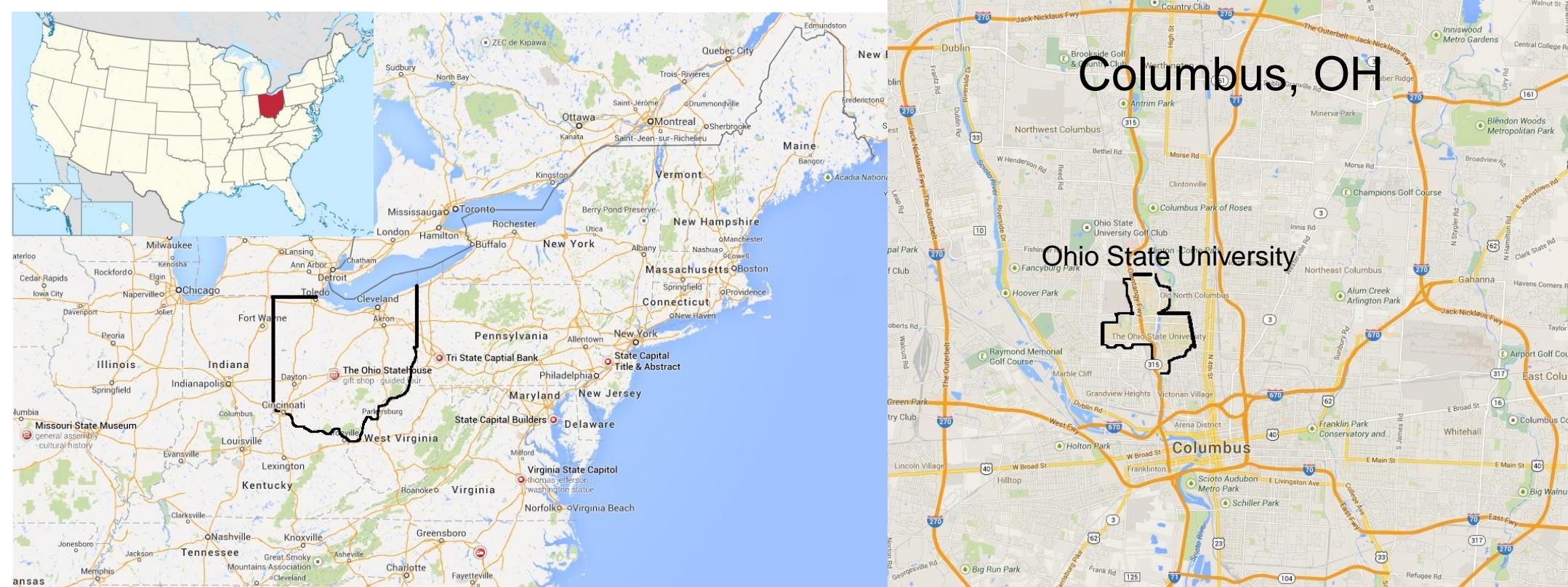
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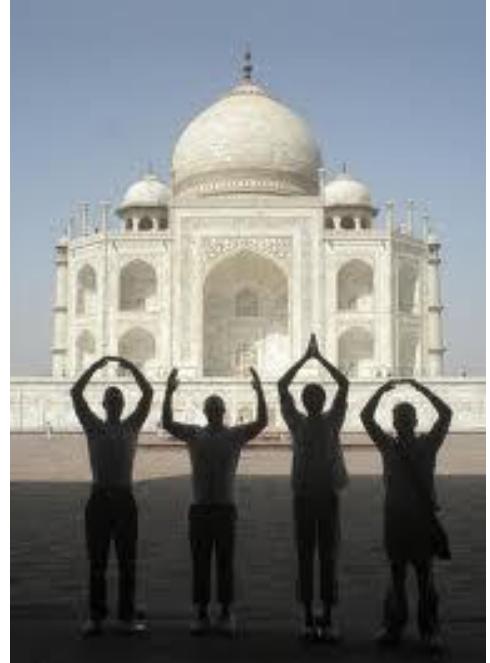
The Ohio State University

Columbus, OH

- Campus 7 km²
- 64,429 Students
- 56,867 Undergraduate
- 13,951 Postgraduate
- 6,254 Academic Staff
- Div I-AAA: 19 mens, 20 womens varsity sports teams
- 475,000 living alumni



O-H-I-O



Disclosure

I have a Research Collaboration Agreement (RCA) with EnvisionTEC, Inc. (Dearborn, MI), a 3D printer manufacturer. I am the lead inventor of patents assigned to, and have received compensation from, and have an ownership stake in, Osteoplastics, LLC (Shaker Heights, OH).



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Overview

- I *Clinical Problem: Large Cranial Defects*
- II Cranial Implant Computer Aided Design (CAD)
- III Light-based polymerization of PPF Scaffolds
- IV Initiators, Dyes, Biologics, Crystals, and Solvents
- V Discussion



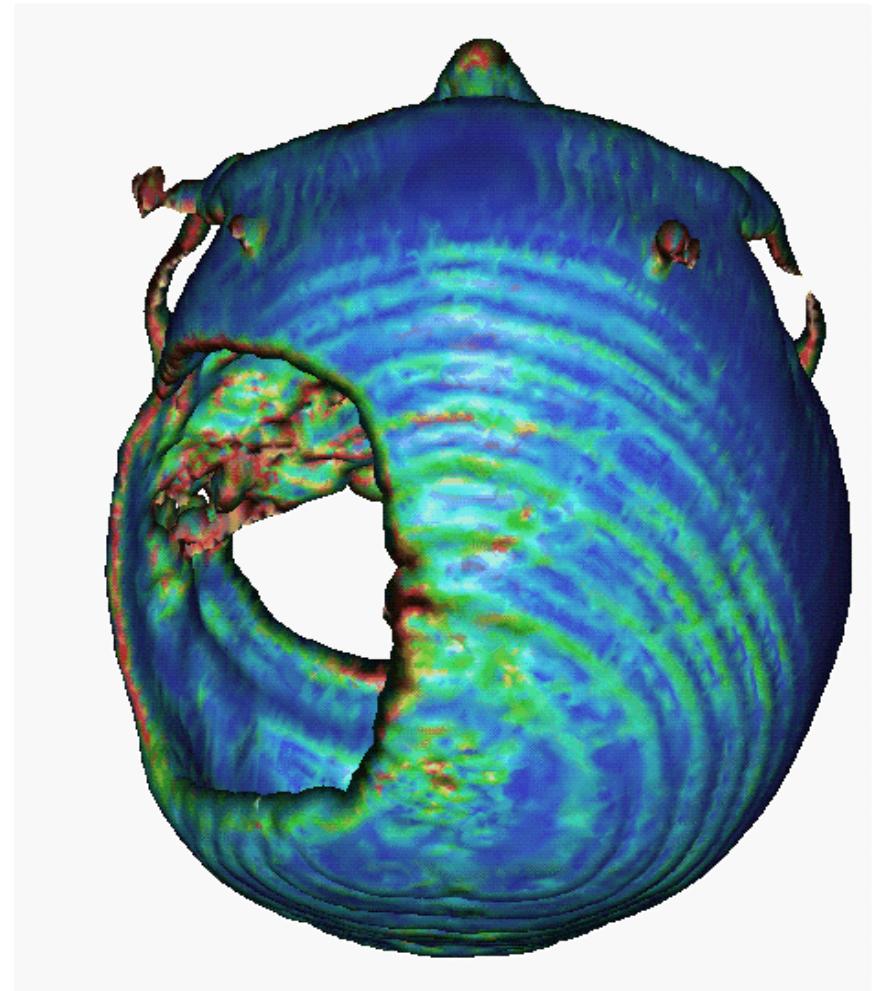
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1.1 Neurocranial Deficit

- Causes: trauma, surgery, cancer.
- Gold standard treatment is autograft which must:
 - Protect brain from trauma
 - Protect brain from infection
- Vascular supply is from dura:
 - Ability to revascularize declines with age
 - Likelihood of deficit increases with age
- Failure to revascularize leads to:
 - Necrosis, resorption, and/or infection



Critical Size Skull Defect

1.2 Engrafting Large Defects

- Autograft (calvarium, rib, ilium, fibula, metatarsal)
 - pain and morbidity at donor site.
 - graft may fail to vascularize.
- Allograft (cadaver bone)
 - risk of disease transmission.
 - graft may fail to vascularize.

1.3 Additive Manufactured Skull Models for Manual Cranial Implant Preparation

- Step one: render a 3D skull model from a 3D CT-scan on a 3D printer.
- Step two: mold a skull plate by pushing clay against the skull model defect site.
- Step three: is to re-cast this skull plate in PMMA implant or bend a titanium plate to fit its contours.

1.4 Pre- and Intra-operatively Malleable Alloplastic Graft Materials

- Manually Determine Cosmesis
- Titanium mesh plates
 - poor barrier to infection.
- Hydroxyapatite Pastes
 - poor tensile strength.
- PMMA
 - exothermic (gives off 180° F).



1.5 Bone Tissue Engineering

- Resorbable polymers
- Bone progenitor (stem) cells
- Growth factors
- A tissue engineered implant, that fit well, might facilitate:
 - host incorporation of the implant
 - graft incorporation and closure will protect from infection
 - remodeling of neobone to cortical bone will protect from trauma
- A tissue engineering approach is already in the clinic for post-cranial applications (e.g., vertebral fusion), however these applications do not use stem cells.
- Computer Aided Design (CAD) is frequently used to prepare inert, not tissue engineered, implants that fit the complex shapes of cranial defects very well.

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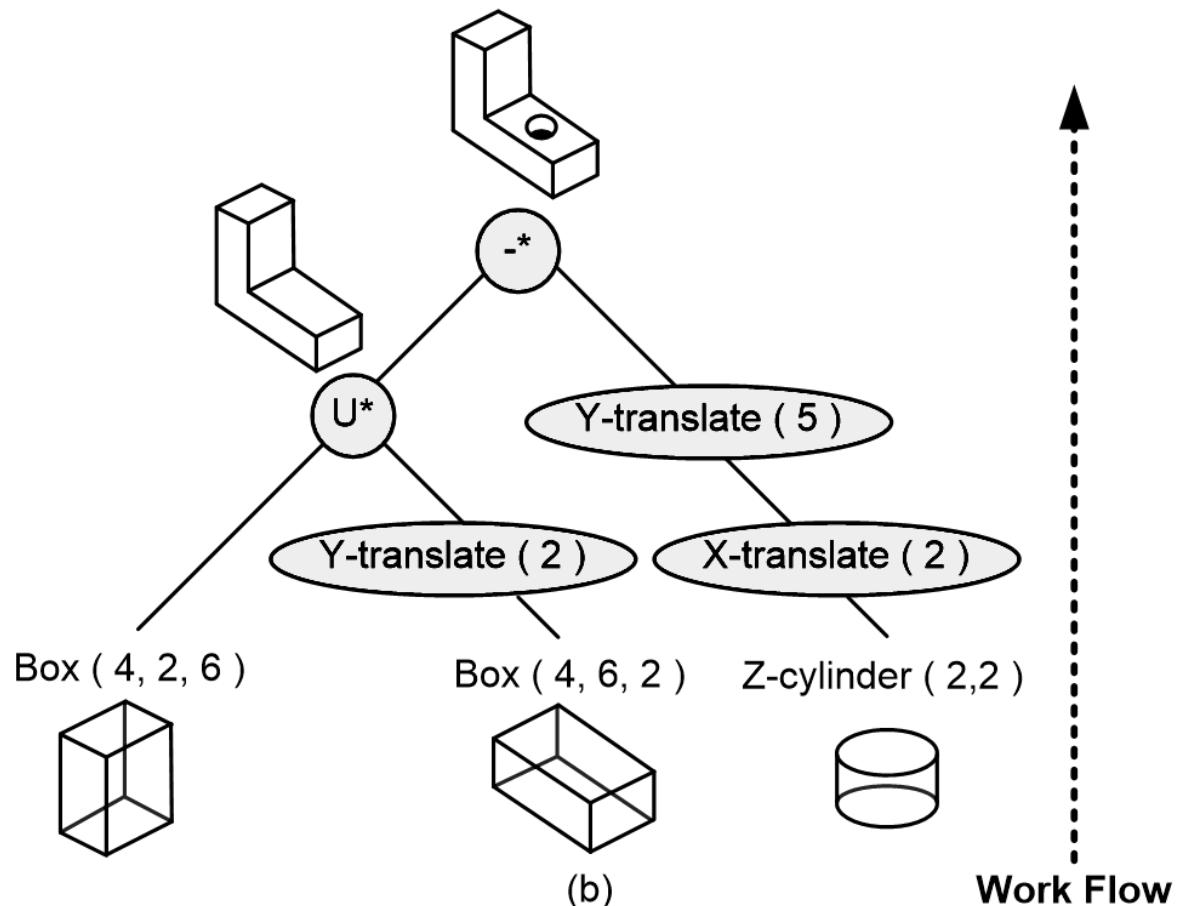
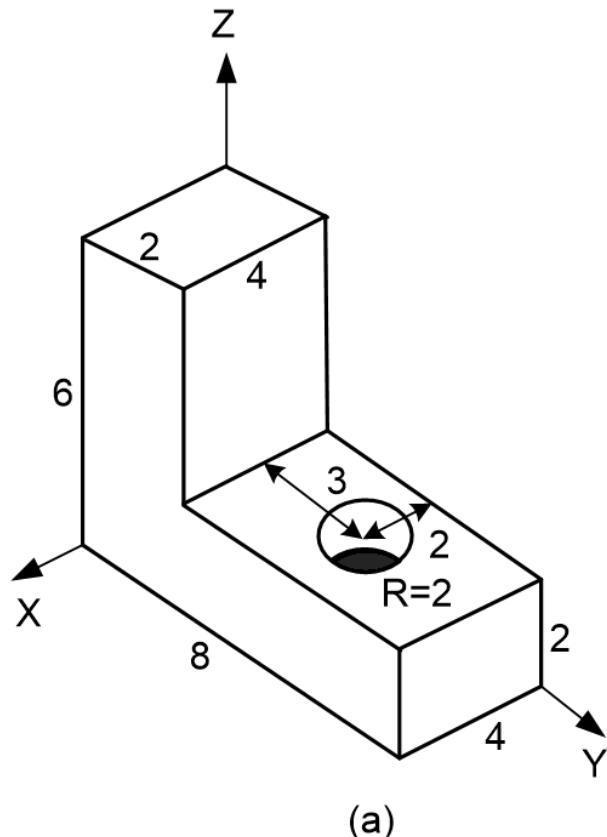
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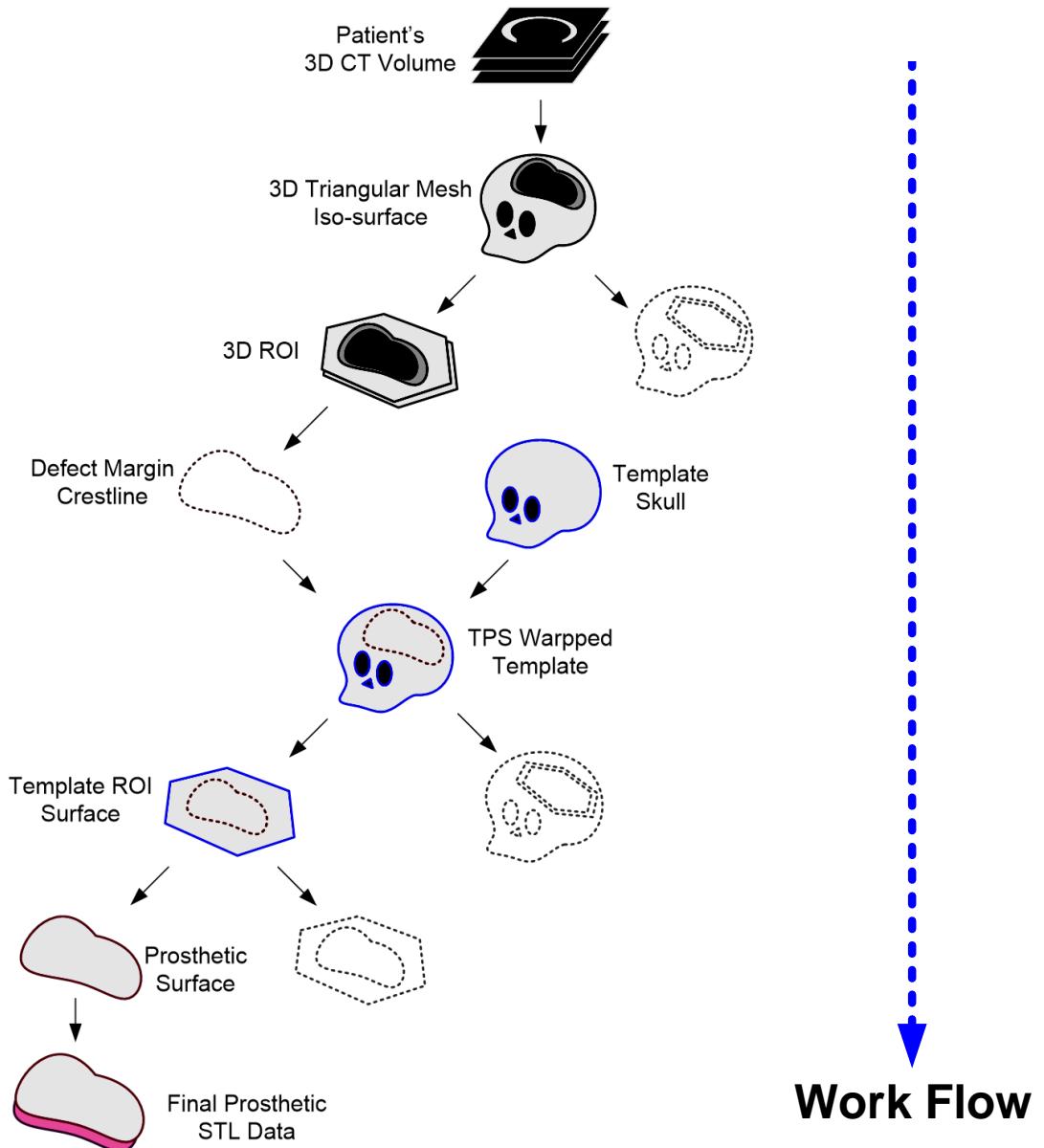
2.1 Bottom-up CAD

- **Bottom-up design approach**

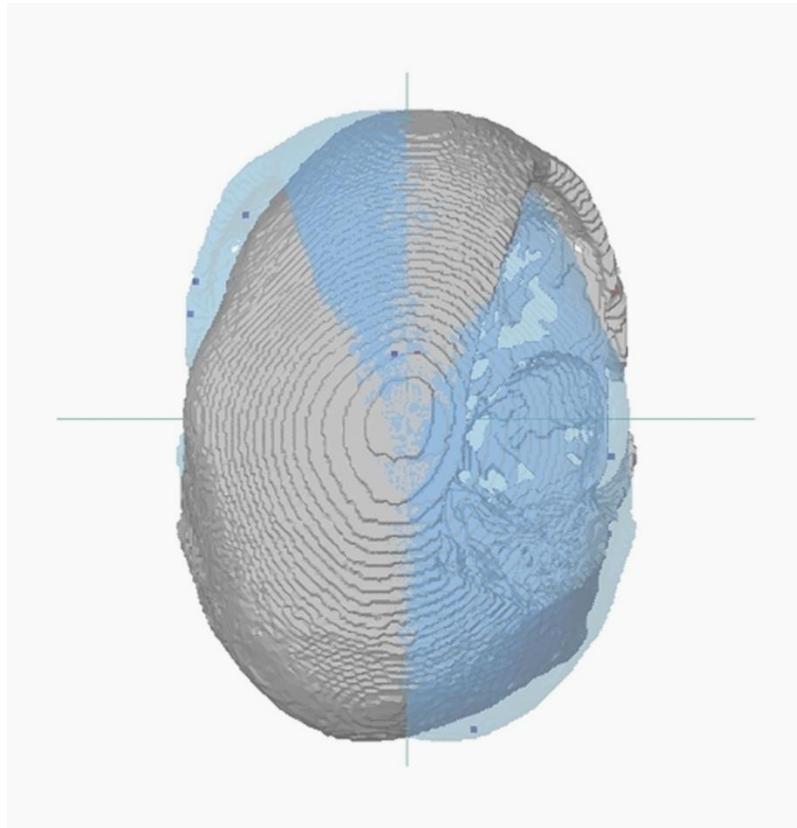


2.2 Top Down Cranial CAD

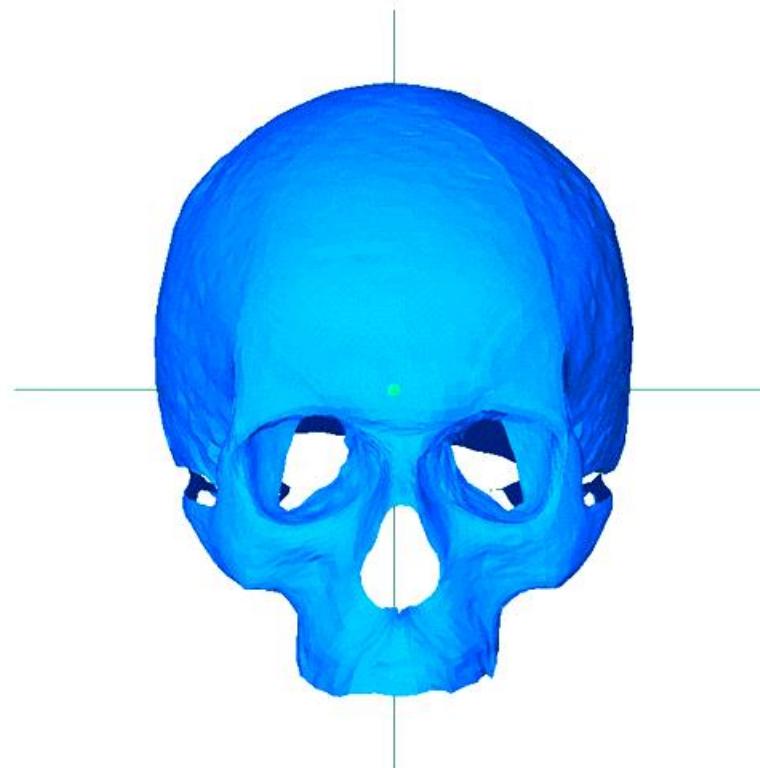
- ① Accurate cranial defect margin detection.
- ② Interpolation of skull surface template into cranial defect.
- ③ Implant surface smoothing if right-left mirrored template.
- ④ Partitioning of implant surface from skull template.
- ⑤ Prosthetic edge and surface fit validation prior to fabrication.



2.3 Unilateral and Bilateral Cranial Defect Skull Templates

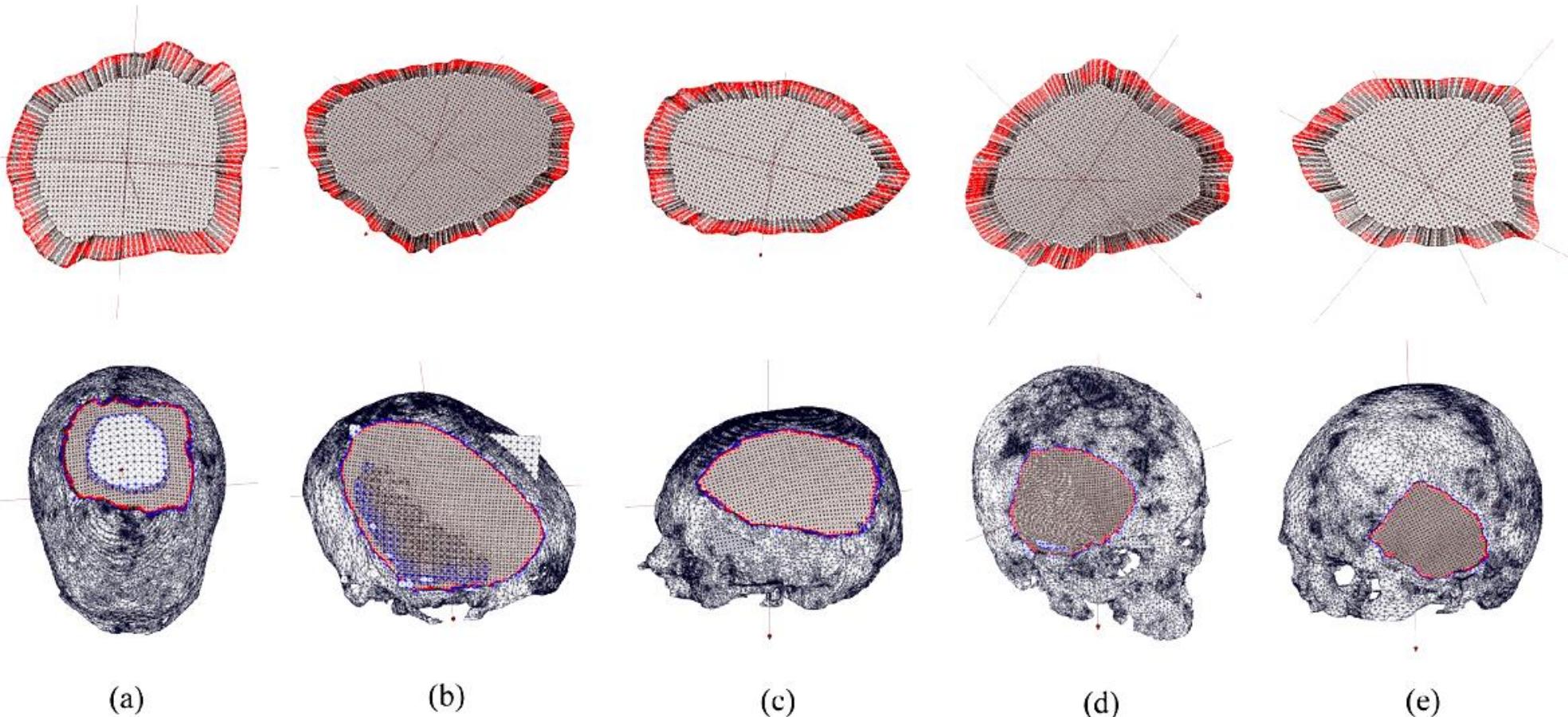


Left-Right Mirrored Skull
Template (Unilateral)

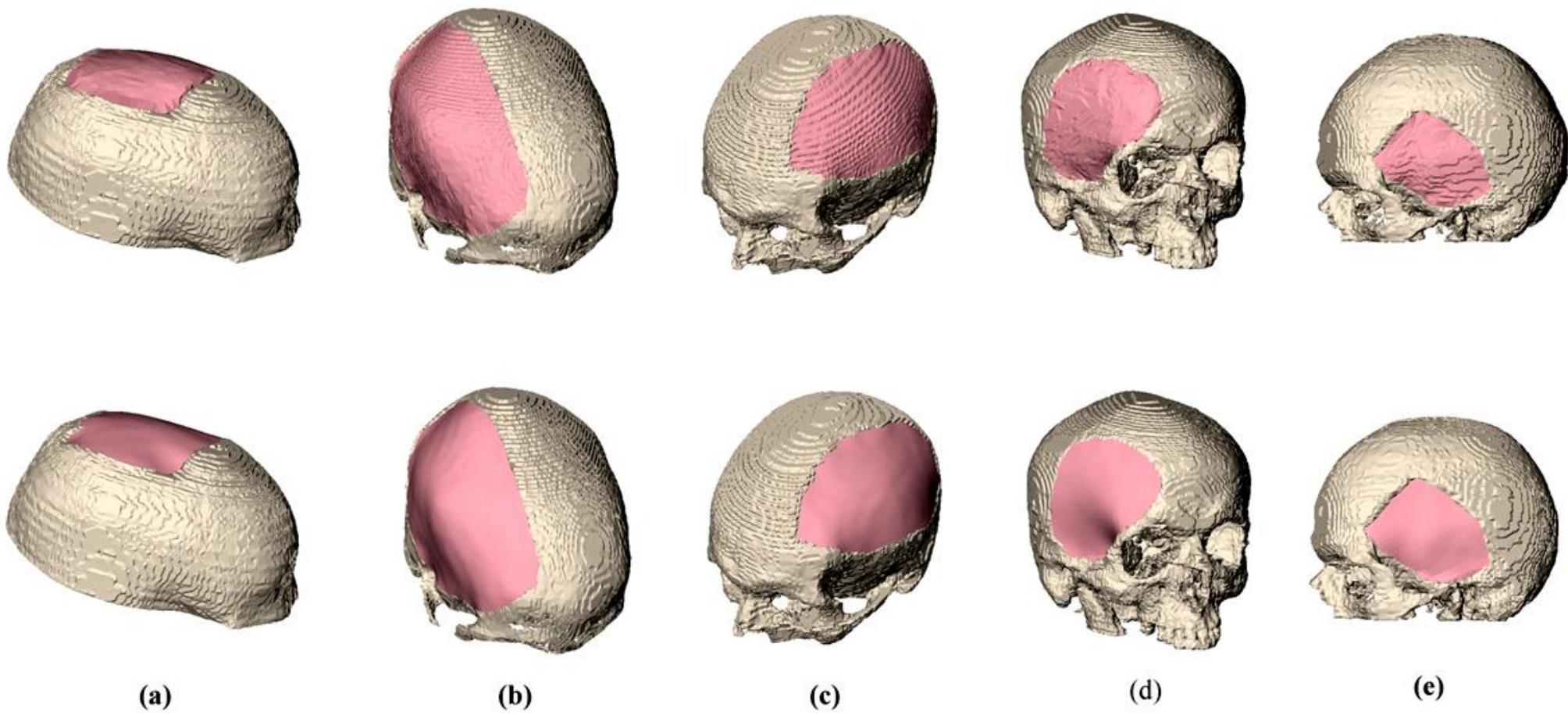


Average Skull Template
(Bilateral)

2.4 Cranial Implant Seating and Cosmesis



2.5 Five Smoothed Cranial Implants



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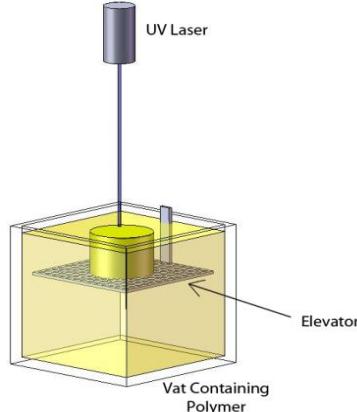
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3.1 Solid Freeform Fabrication (SFF) Technologies:

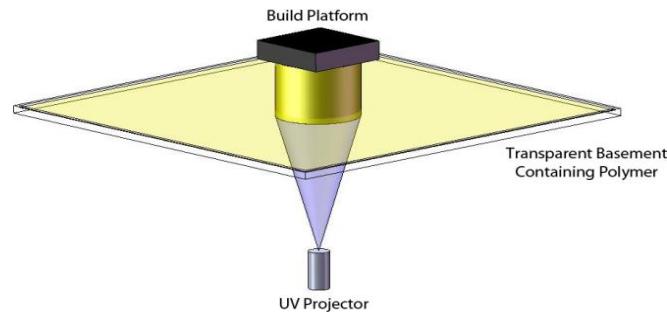
“This new paradigm requires scaffolds that balance temporary mechanical function with mass transport to aid biological delivery and tissue regeneration.”
(Hollister SJ (2005) Porous Scaffold Design for Tissue Engineering. Nat Mater 4:518-524).

3.2 Additive Manufacturing

- “[The] process of joining materials to make objects from 3D model data, usually layer upon layer...” –ASTM F2792-10
- Two additive manufacturing (AM) methods have very high accuracy
- Both use ultraviolet light to photo-crosslink resorbable polymeric resins:



Stereolithography



Continuous DLP Rendering



3D Systems Viper® HA
SLA device



envisionTEC Perfactory®
cDLP device

- Stereolithography (SLA) and Continuous Digital Light Processing (cDLP) have both been used to polymerize poly(propylene fumarate) (PPF).

4.1 envisionTEC Perfactory

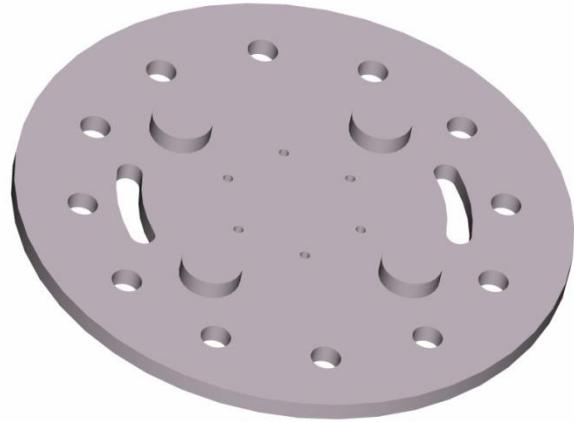


<http://www.envisiontec.com/fileadmin/images/PerfMovieRC25.swf>

3.3 Additive Manufacturing: Why Poly(propylene fumarate)?

- Controllable degradation releases small amounts of fumaric acid:
 - Does not bulk degrade
 - Does not cause acidic spike
 - metabolized in Krebs cycle
- Degradation control via:
 - Cross-linking density (stiffness)
 - molecular weight (strength)
- Prior work with translucent molds.
- Can be UV light polymerized in the presence of a photoinitiator.

3.4 CWRU PPF SLA System



CAD implant design:

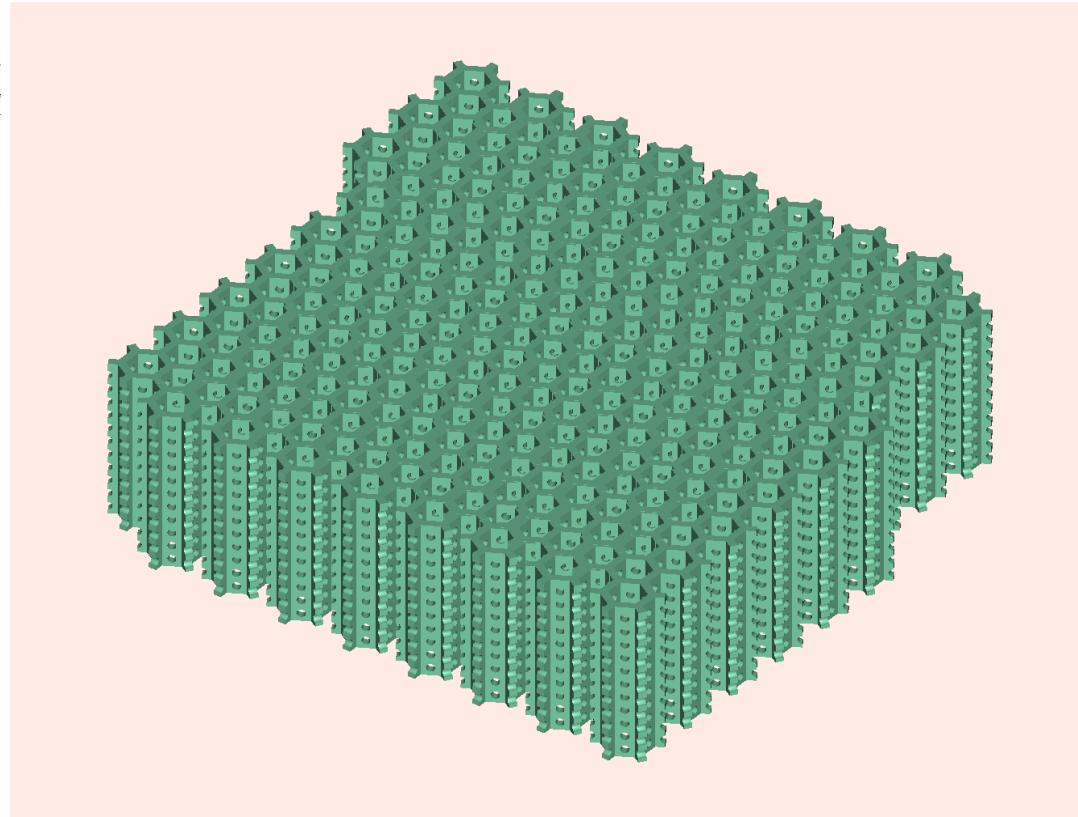
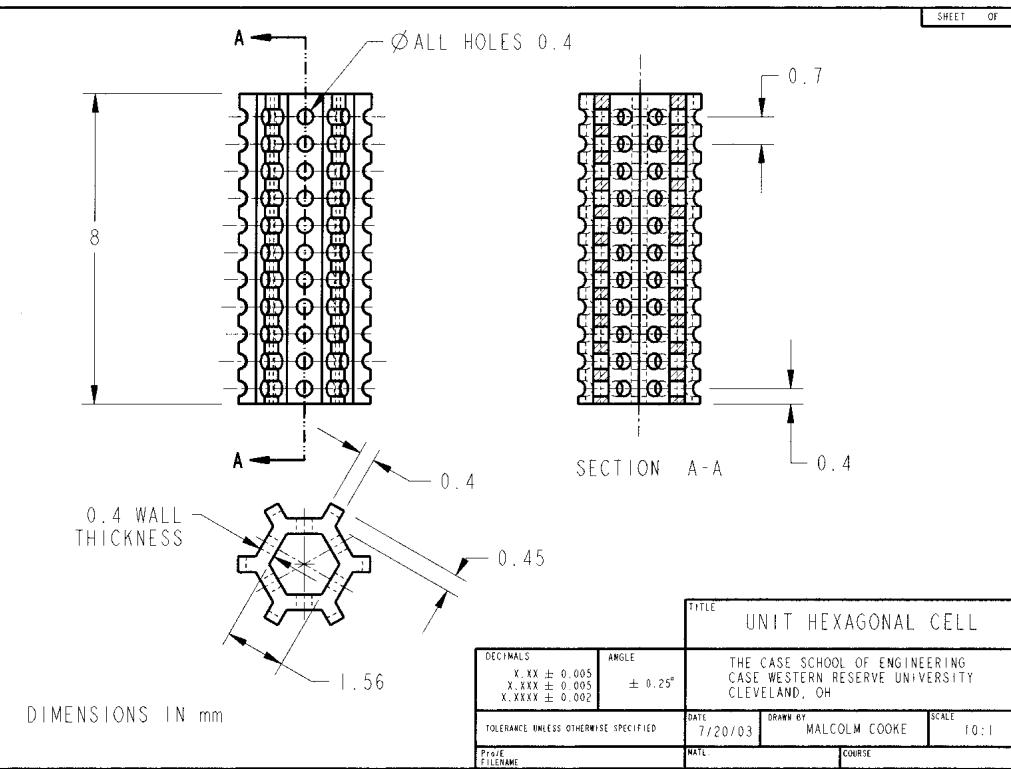
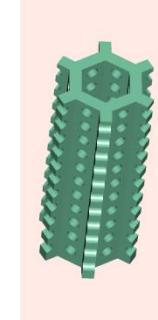
- Designed in ProEngineer™.
- Dimensions are 50mm diameter x 4mm thick.

CAM implant output:

- Complete dimensional accuracy.
- Some support structures failed to build.

3.5 Control Porous Resorptive Fronts

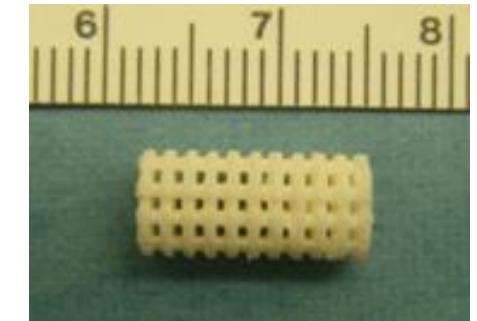
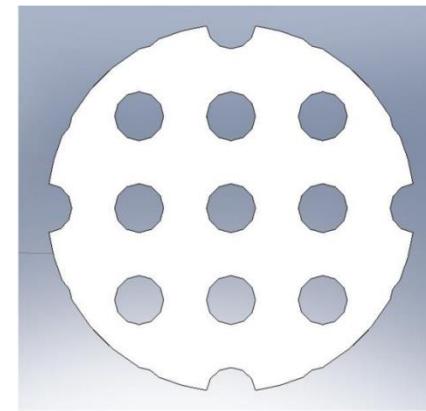
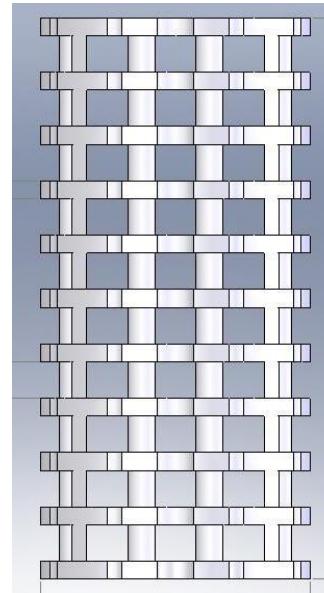
- Design porous margins of cranial implants to promote bony ingrowth.



3.6 Test Scaffold: “Plate and Post” Geometry

Implant Design

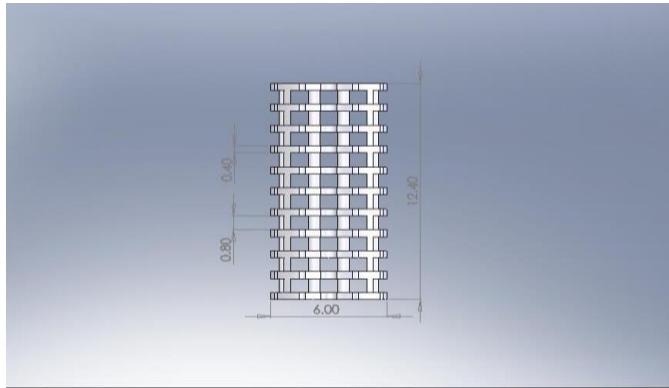
- Highest accuracy in Z with SLA was 400 microns.
- 800 μm diameter vertical channels.
- 400 μm thick plates with 800 μm gaps.
- Large vertical channels facilitate scaffold loading and vasculature.
- Inter-plate spaces open toward defect margin to facilitate tissue in-growth in cranial model.
- Use cylinder in athymic nude rat model with allogenic cells.



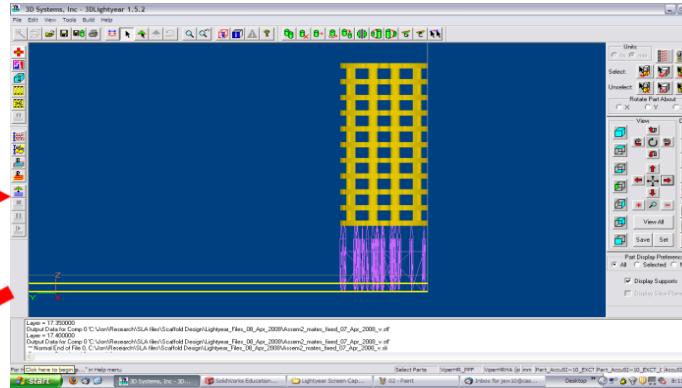
cDLP- rendered scaffold

CAD file

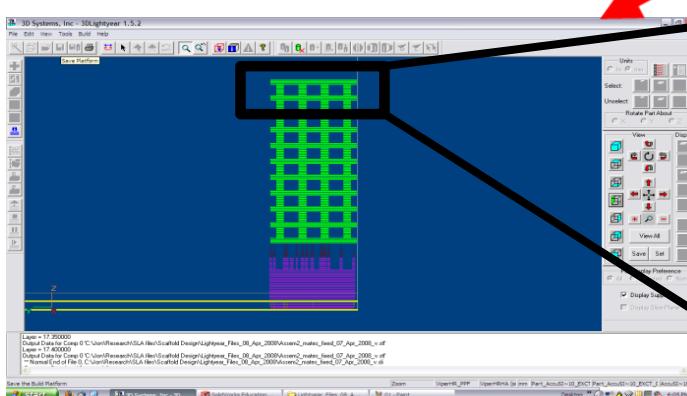
3.7 Preparing the Design for Additive Manufacturing



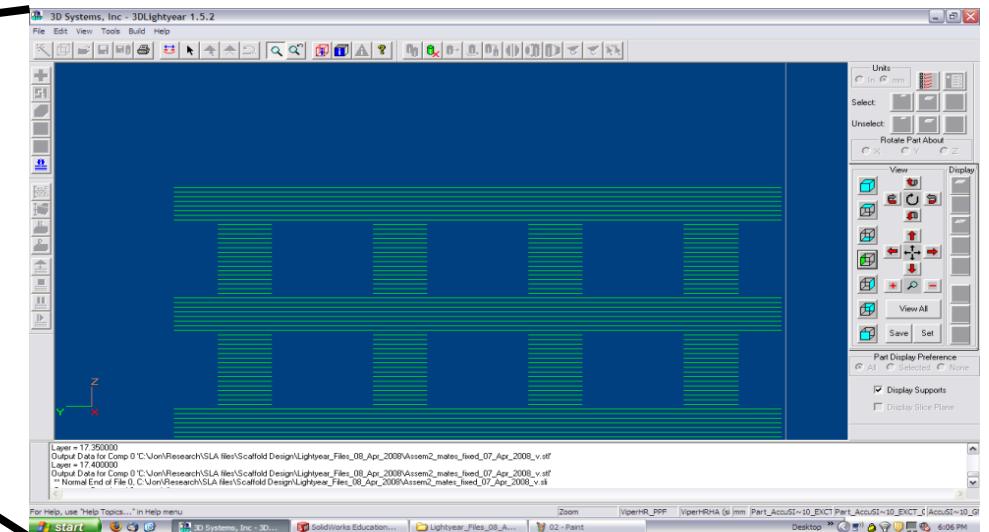
Create design in CAD software and convert to STL file.



Create Support Structures.

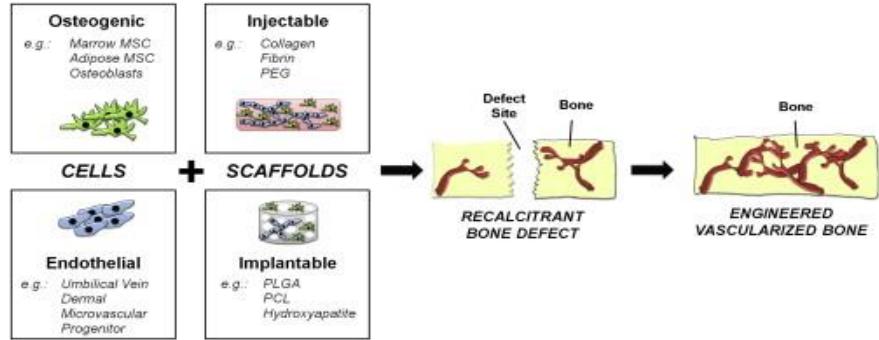


Slice the STL file into layers to create a Build File.

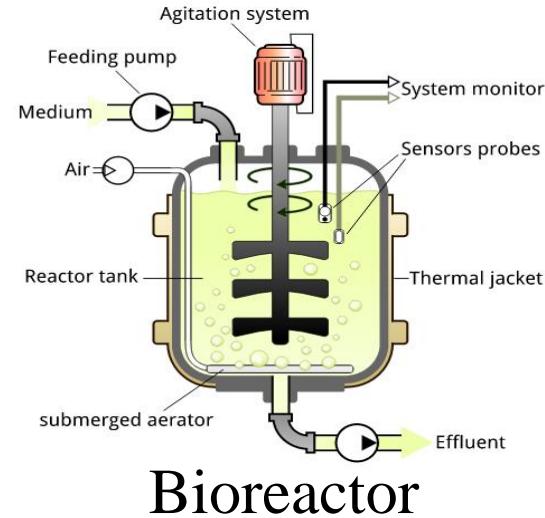


3.8 Applications: Tissue Engineering

- Scaffold
- Stem Cells
- Growth Factors
- Bioreactor
- Example:
 - Cranial Flap Regeneration:
 - Design Scaffold
 - AM of design in resorbable material



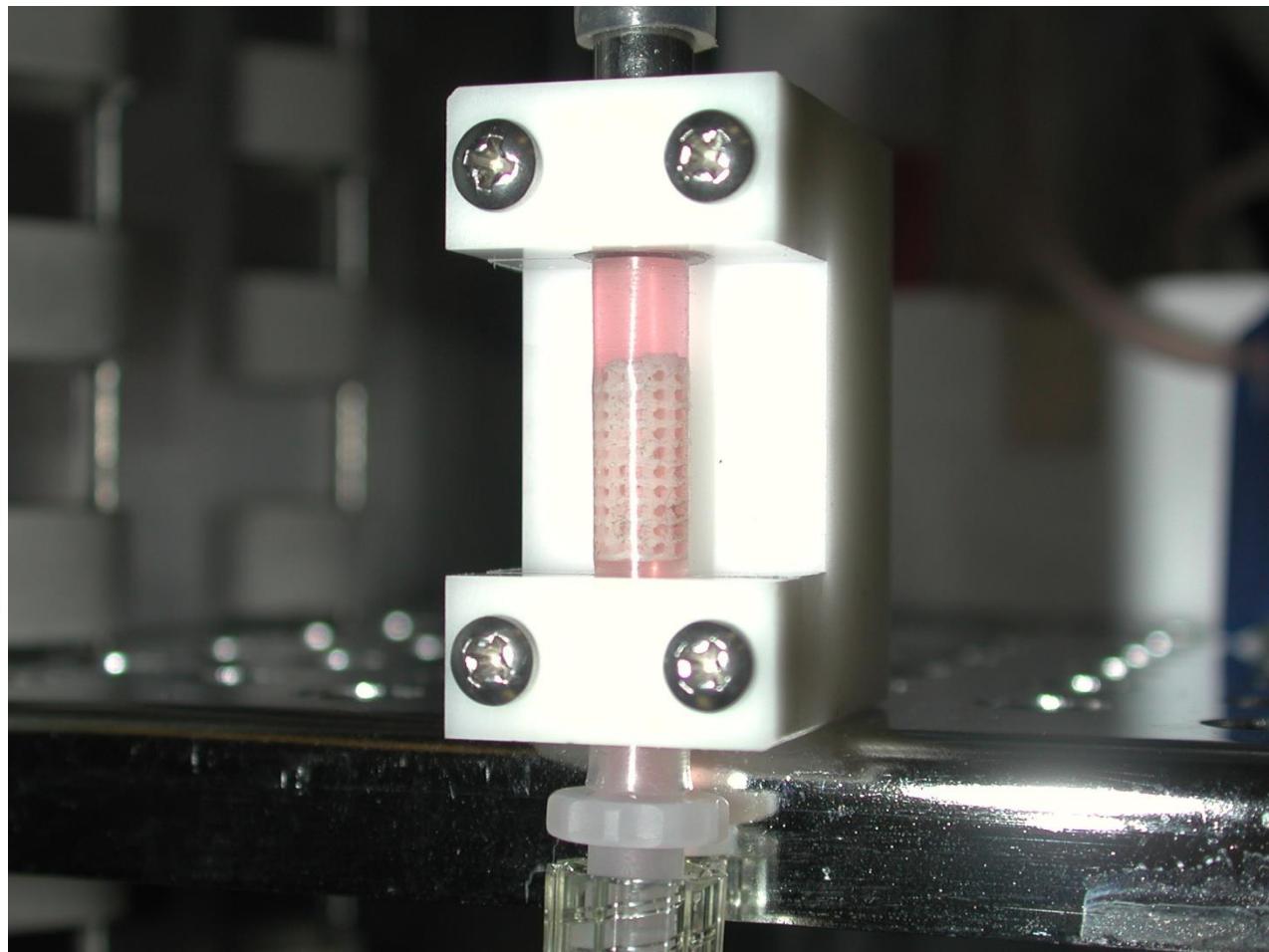
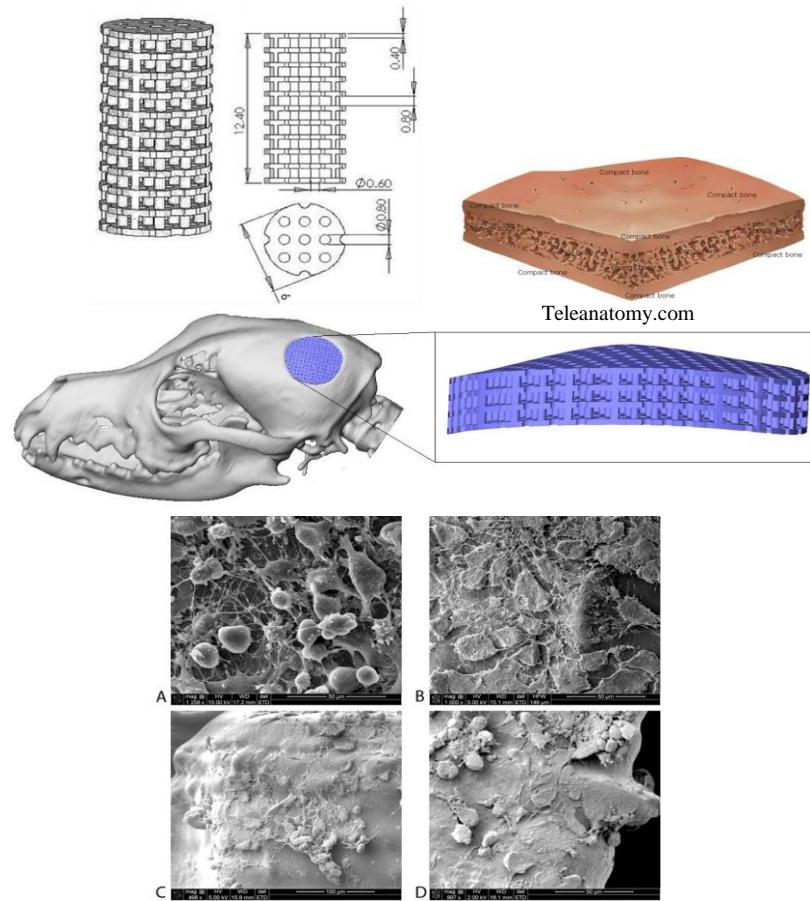
Rao RR, Stegemann JP. Cell-based approaches to the engineering of vascularized bone tissue. Cyotherapy 0:1-14, 2013, p. 5



http://upload.wikimedia.org/wikipedia/commons/e/ee/Bioreactor_principle.svg

Bioreactor

- MSCs Seeded on Resorbable Polymer Implant.



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4.1 Initiator, Dyes/Biologics, Crystals, and Solvents

- Polymerization Initiation: Photo-initiator (0.5-2.0%): bis(2,4,6-trimethylbenzoyl) phenylphosphine oxide (BAPO) Irgacure 819 or Irgacure 784.
- Depth of polymerization: Used pharmaceuticals, TiO_2 , and benzophenone (HMB).
- Surface Roughness: 3D-printed PPF is hydrophobic and smooth. Add HA or b-TCP

4.2 Solvent for High Viscosity

- PPF is extremely viscous, may not flow in 3D printing
- Higher molecular weight PPF provides good bone ingrowth, but does not always fully resorb.
- We have studied low molecular weight PPF.
- One could also make features thinner to insure resorption.
- Additive Manufacturing allows control of both external shape and internal pore geometry.
- DEF is incorporated in the scaffold, reducing rigidity.
- Strength reduces dramatically between 50-75% DEF

4.3 cDLP Improves Resolution and Green Strength

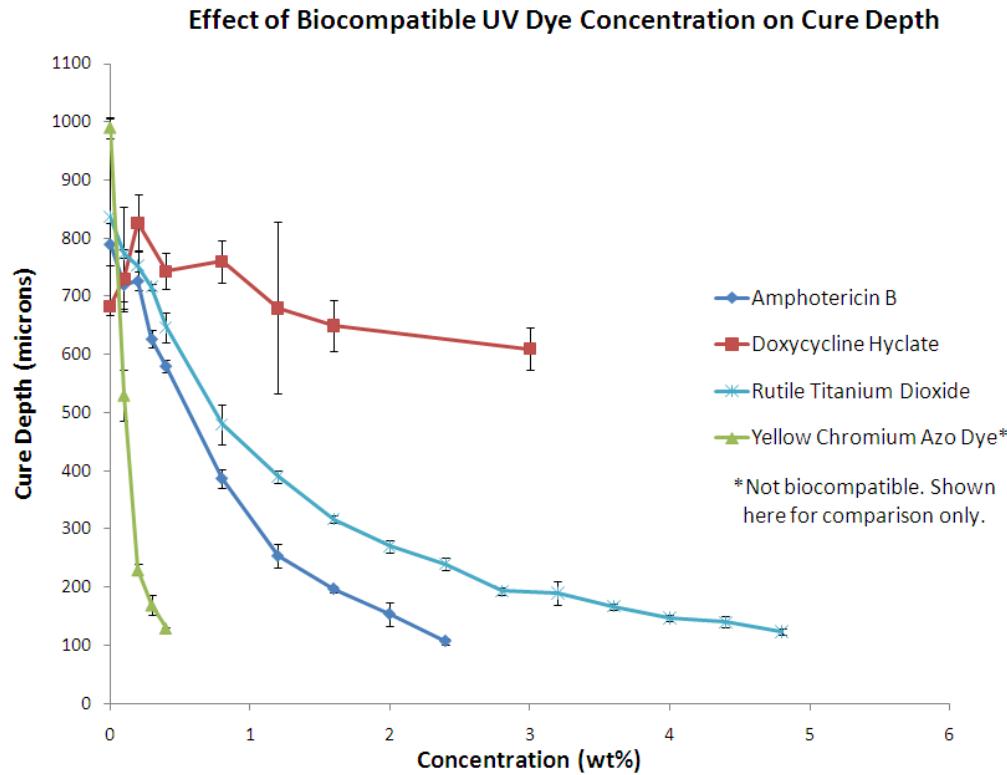
- Perfectory® UV device with 60 mm lens
 - native 71 μm in-plane (x,y) resolution
 - 35.5 μm in-plane (x,y) resolution with anti-aliasing
- Yellow azo chromium dye was added to PPF
- Photo-polymerization initiator, Irgacure® 819 (BASF (Ciba, Florham Park, NJ), BAPO, was added to polymer
- Diethyl fumarate, the monomer precursor of PPF, was added as a solvent in a 1:1 ratio to PPF
- Between plane (z) voxel height 120 μm rendering designed 50 μm layers
 - 200 mW/dm² irradiance
 - layer exposure time of 300 s.
 - Design is 50 μm layer thickness
 - Rendered with 120 μm thick layers

4.4 Dye-Initiator Packages

- Dye competes with initiator.
- Dye creates maximum depth of penetration regardless of energy input used.
- Three types of dyes:
 - UV absorber
 - UV blocker
 - UV scatterer
- Like monomer (DEF), dye is incorporated into scaffold, therefore, dye must be biocompatible.
- Sufficient solubility to insure properties do not change during build cycle.

4.5 Biocompatible Dyes

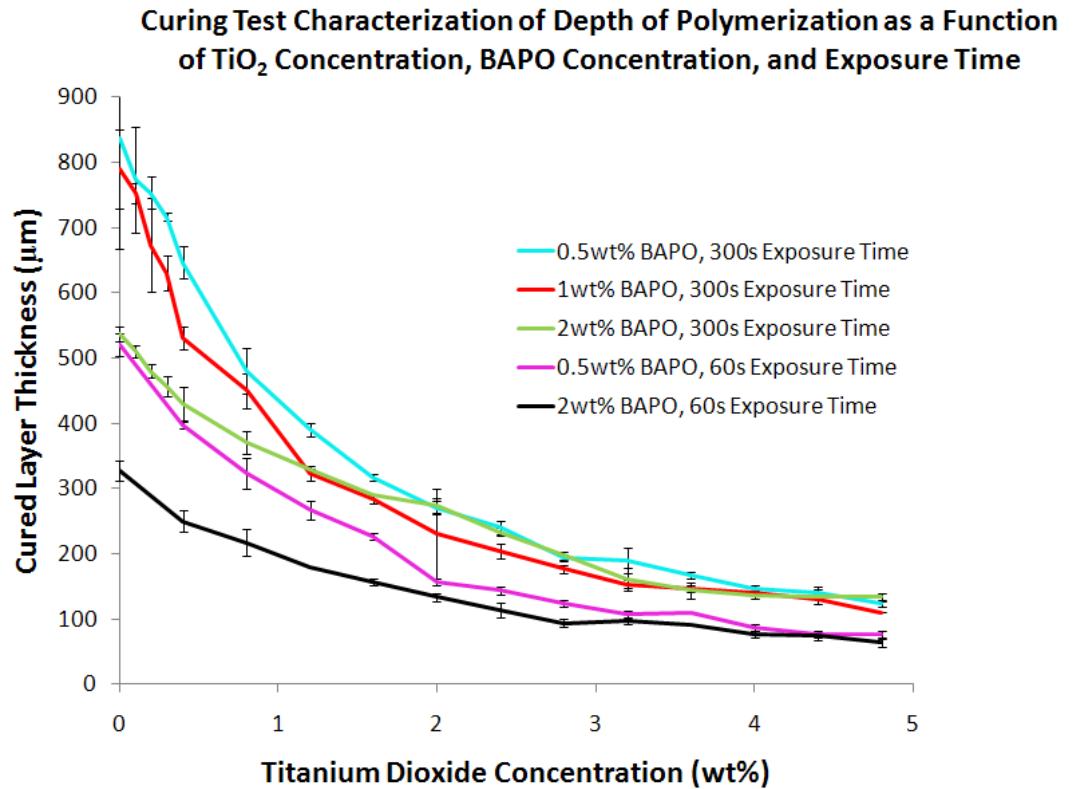
- Azo Chromium dye
120 μ @ <1%
- Potential secondary properties of dyes
 - Extracellular Matrix Component
 - Surface Roughness (particulate)
 - Buffer pH
 - Color
 - Antibiotic
- Antibiotics
 - Doxycycline Hyclate < 600 μ
 - Amphotericin B 120 μ but < 2%
 - Titanium Dioxide (rutile) 120 μ but lateral overcure



The relationship between cure depth (μm) and concentration (wt%) for three biocompatible dyes (amphotericin B, doxycycline hyclate, and rutile titanium dioxide).

4.6 TiO₂ (rutile)

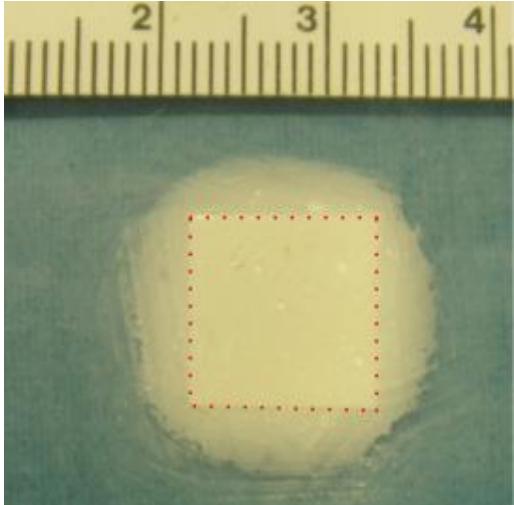
- USP and FCC sources available
- Our source:
Sachtleben (White Plains, NY) 320 nm crystals
- Little affect above 2% BAPO concentration



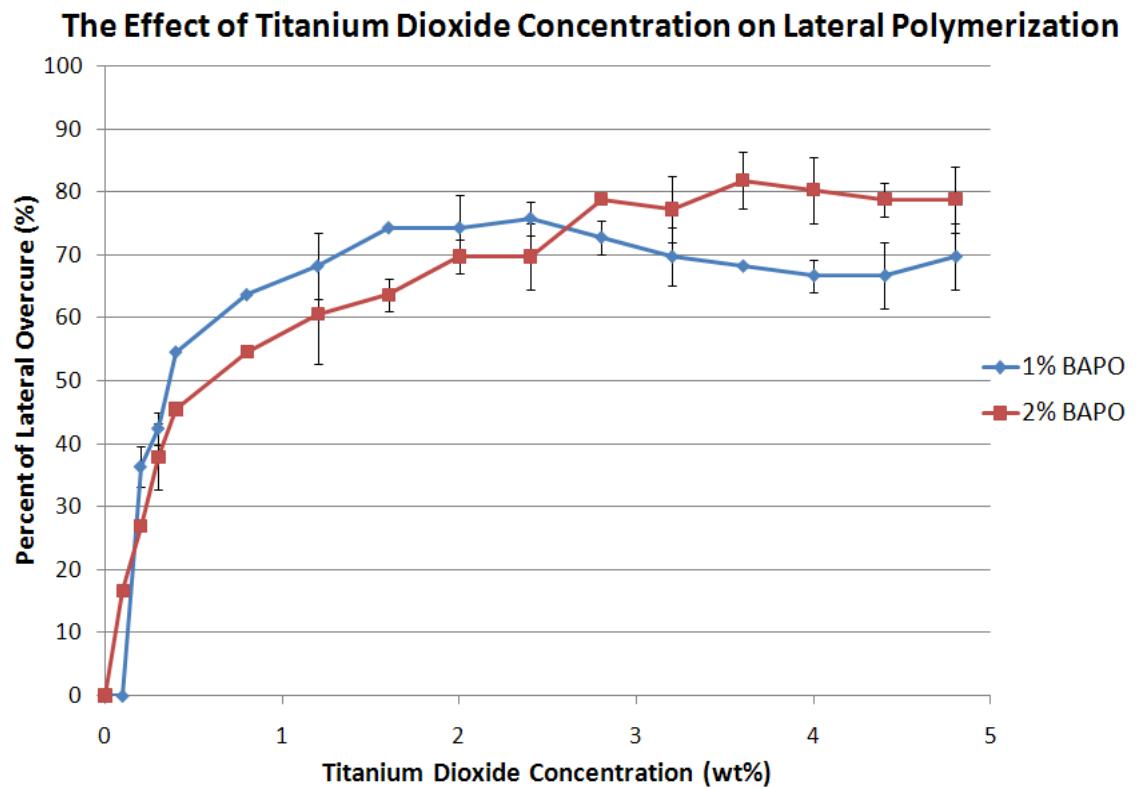
Depth of polymerization (μm) was characterized as a function of titanium dioxide concentration (wt%) for five different combinations of BAPO concentration (wt%) and exposure time (s = seconds).

4.7 TiO₂ Lateral Overcure

- Lateral overcure
- Minimized below best concentration for BAPO (2%)
- May have little affect on build accuracy



Lateral overcure measurement.



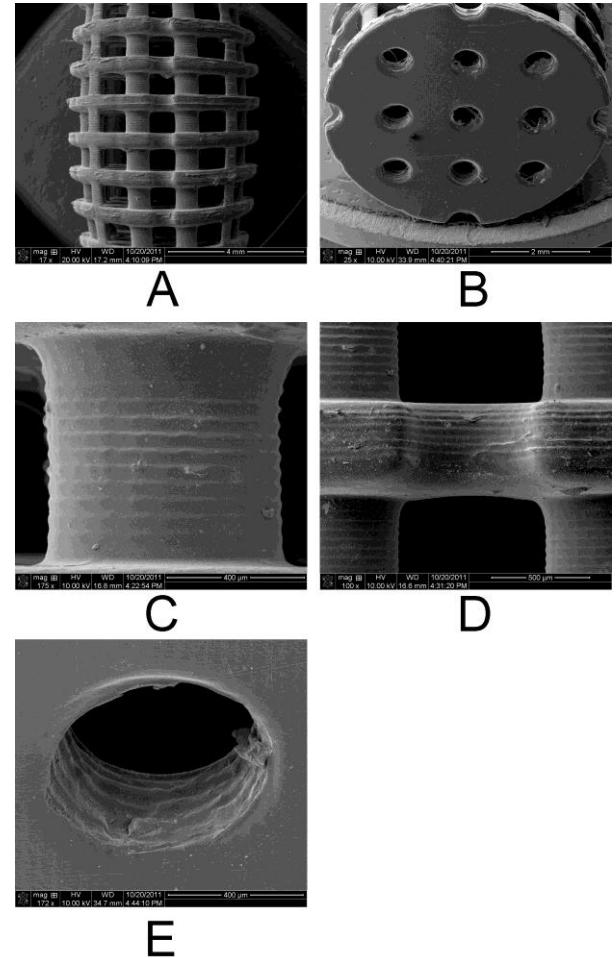
Increasing titanium dioxide concentration led to an increased amount of lateral overcuring.

Other dyes? I819 + I784 + HMB?

- Accuracy?
- Pre- to post-cure strength?
- Table?

TiO₂ Allows High Accuracy

- SEM of plate and post scaffolds
- Feature thickness accuracy within 6.2 μ
- Curved feature inaccuracy 5.8-35.8%

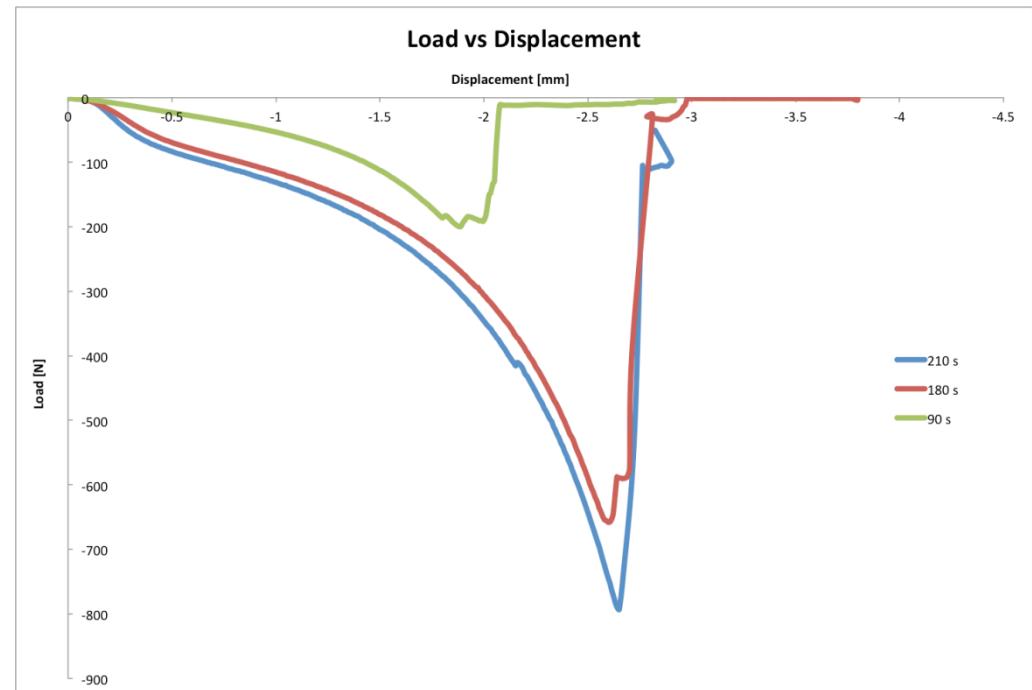


Tuning Resin Strength

Mechanical Testing

Exposure Time	Layer Thickness	Average Modulus (N)
90 sec	120 mm	33 MPa (1)
180 sec	150 mm	166 MPa (3)
210 sec	180 mm	195 MPa(5)

- Must be able to clean pores prior to post-processing
- Must be able to handle surgically following post-processing



Surface Roughness

- 100 nm, 1 m, 10 m, 100 m
- Quantify features in RMS roughness and height map
- Affect on MSC attachment and proliferation
 - Cells perceive mechanically
 - Cells perceive chemically
 - Which are determinant at different scale?

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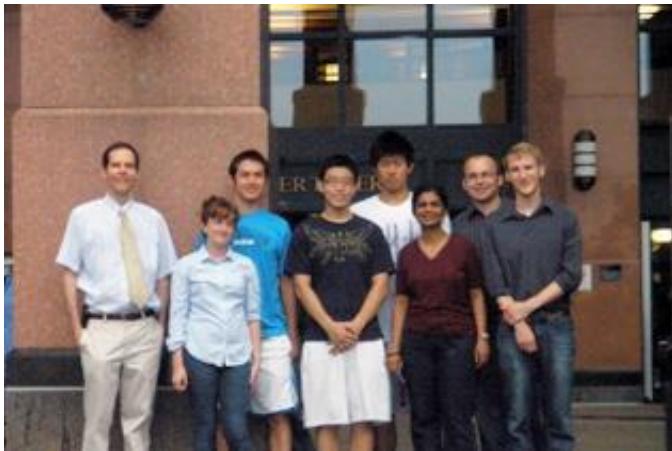
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5.1 Discussion

- Secondary uses for dyes or other resin solutes:
 - Extracellular Matrix Component
 - Surface Roughness (particulate)
 - Buffer pH
 - Color
 - Antibiotic
- biocompatible dye that is FCC or USP grade.
- Exposure time (energy) affects layer thickness and strength.
- Delamination is a problem with insufficient curing.
- Too much time leads to basement plate adhesion.

5.2 Future Work

- Light sources (mercury lamp vs LED)
- Peeling mechanism?
- Basement plate adhesion?



Imaging Laboratory Department of Neurological Surgery

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Project Collaborators:

PPF Synthesis:

- Martha Wang
- Kyobum Kim
- John Fisher

Scaffold Rendering and Imaging:

- Jonathan Wallace

Sage Advice:

- Arnold Caplan
- Al Siblani
- Tony Mikos
- Warren Selman



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Thank you for your attention. Comments or Questions?

Acknowledgements:

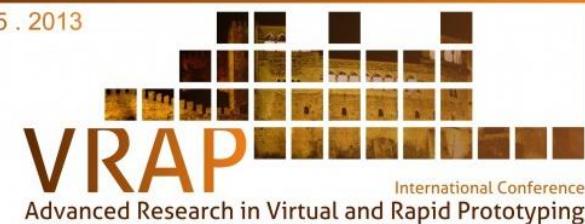
- Research Foundation of the Department of Neurological Surgery, CASE/UHC
- National Institutes of Health grant R01-DE013740
- Clinical Tissue Engineering Center, Cleveland, OH

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October 1 to 5 . 2013



October 4, 2013