Building Bone with Polymers: How new Materials & additive manufacturing are changing medicine

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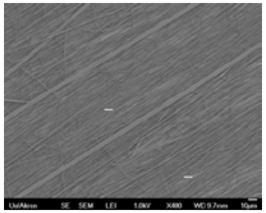
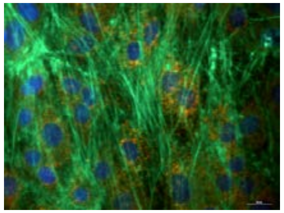
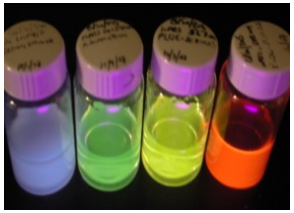
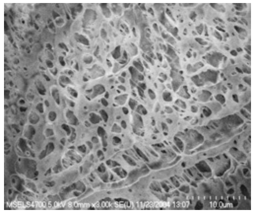
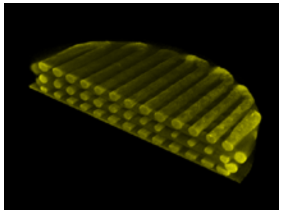
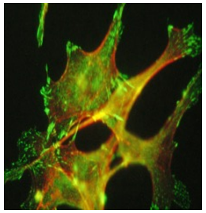
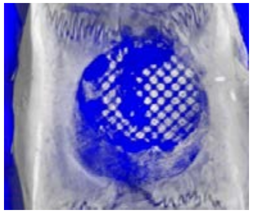

BECKER LABORATORY FOR FUNCTIONAL BIOMATERIALS

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Air Force– Trust Initiative

Ohio Third Frontier – Innovation Platform, Ohio Opioid Challenge

Merck, Cook Biotech, Cook Medical, Viscus Biologics,
21st Century Medical Technologies
Knight Foundation, Kepley Foundation

The Becker Group

to develop translationally-relevant chemical methods that enable the Regioselective placement of bioactive species on polymers

Segmental Bone Defect Repair

CMF Reconstruction

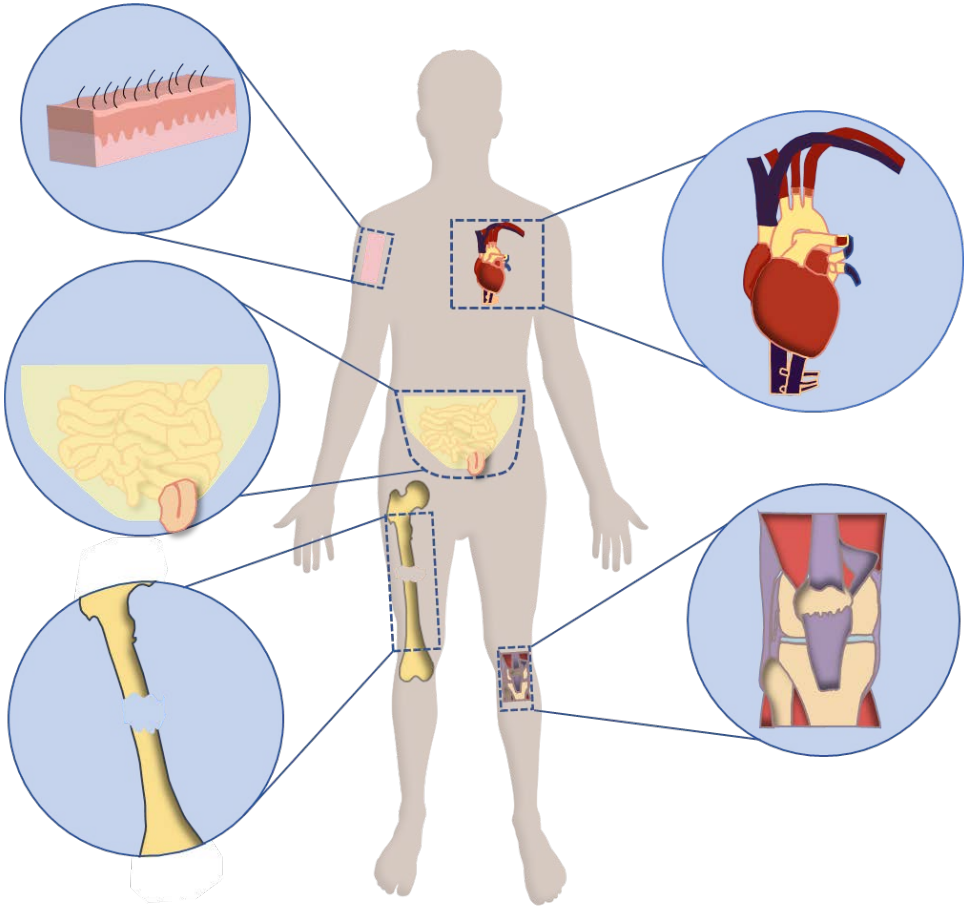
Peripheral Nerve Defects

Tissue Engineered Vascular Grafts

Anti-microbial Materials

Drug Delivery

Hernia Repair



Molecular Recognition

Catalyst Design

Small Molecule Synthesis

Methodology

Ring Opening Polymerization

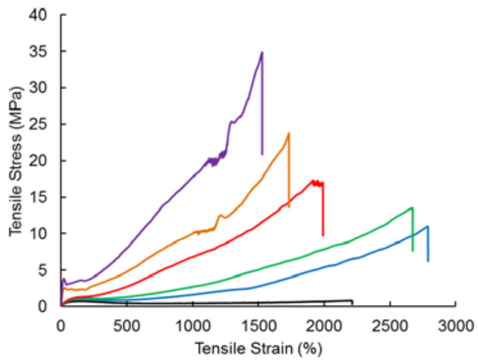
Bioconjugate Design

Molecular & Cell Biology

Chemical innovations motivated by unmet medical needs

The Becker Group

Degradable Elastomers

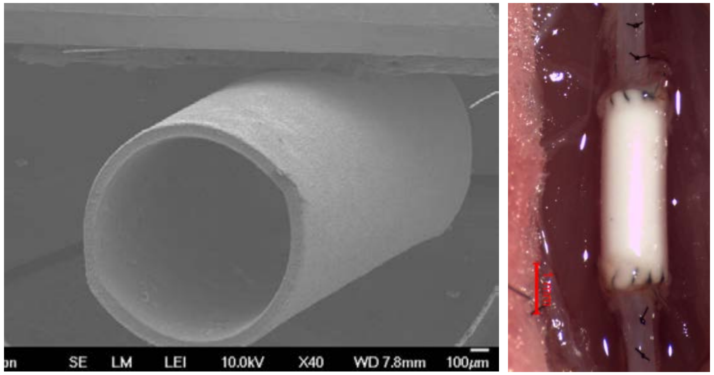


Angew Chem. Int. Ed. 2016, *55(42)*, 13076-13080.

Hydrogels

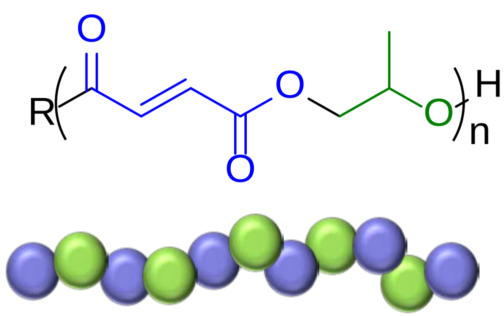
Advanced Materials, 2015, *27 (40)*, 6283-6288

Tissue Engineered Vascular Grafts



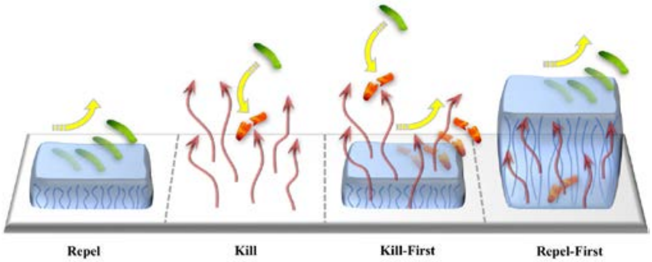
Advanced Healthcare Materials, 2016, *5(18)*, 2427-2436.

ROP Catalysis



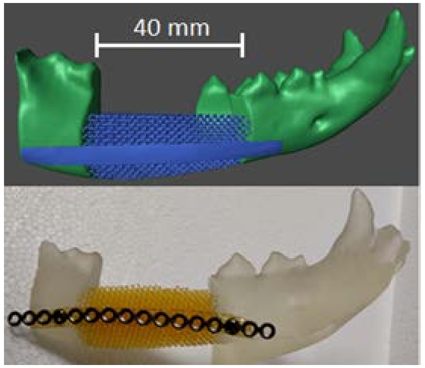
J. Amer. Chem. Soc. 2018, *140* (1), 277–284

Antimicrobial Materials



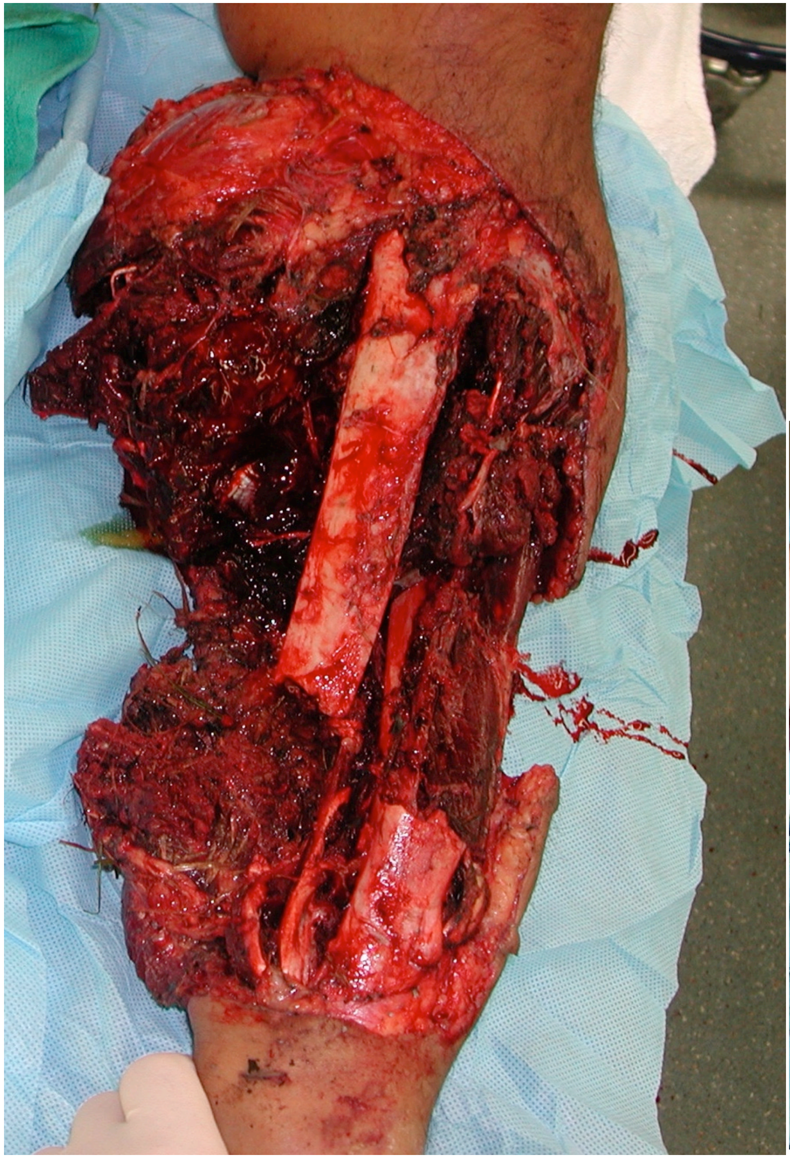
ACS Macro Lett., 2018, *7*, 16-25.

Additive Manufacturing



Biomaterials 2017, *141*, 176-187.

The problem we are trying to address



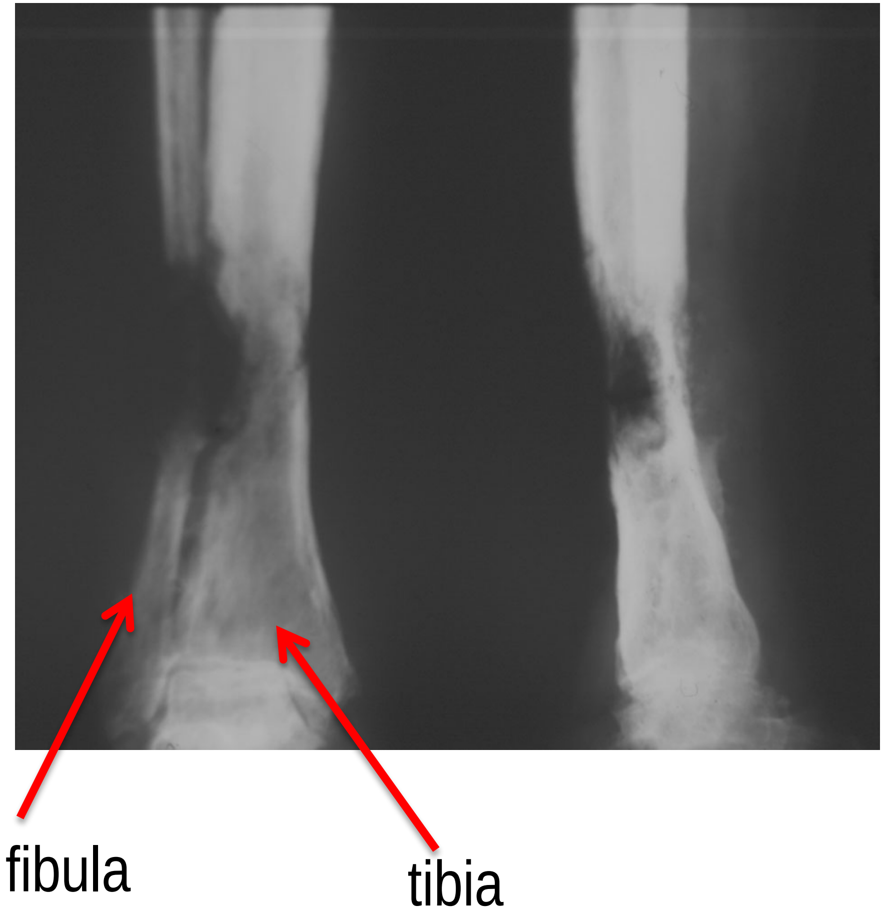
56% of war-injured patients have sustained blast-related injuries (e.g., IEDs or RPGs)

Overall Casualties in OIF, OND, and OEF, 2001-2014	
U.S. Service member Deaths	4,412
U.S. Service members Wounded in Action	31,949
Traumatic Brain Injuries	>150,000

Photos from Ennio Tasciotti, HMRI

Unmet Medical Need - Segmental Bone Defects

>1.9M bone graft procedures in 2012
~200,000 critical sized defects



Acute limb shortening
- Inappropriate for large defects
- Potentially poor functioning result

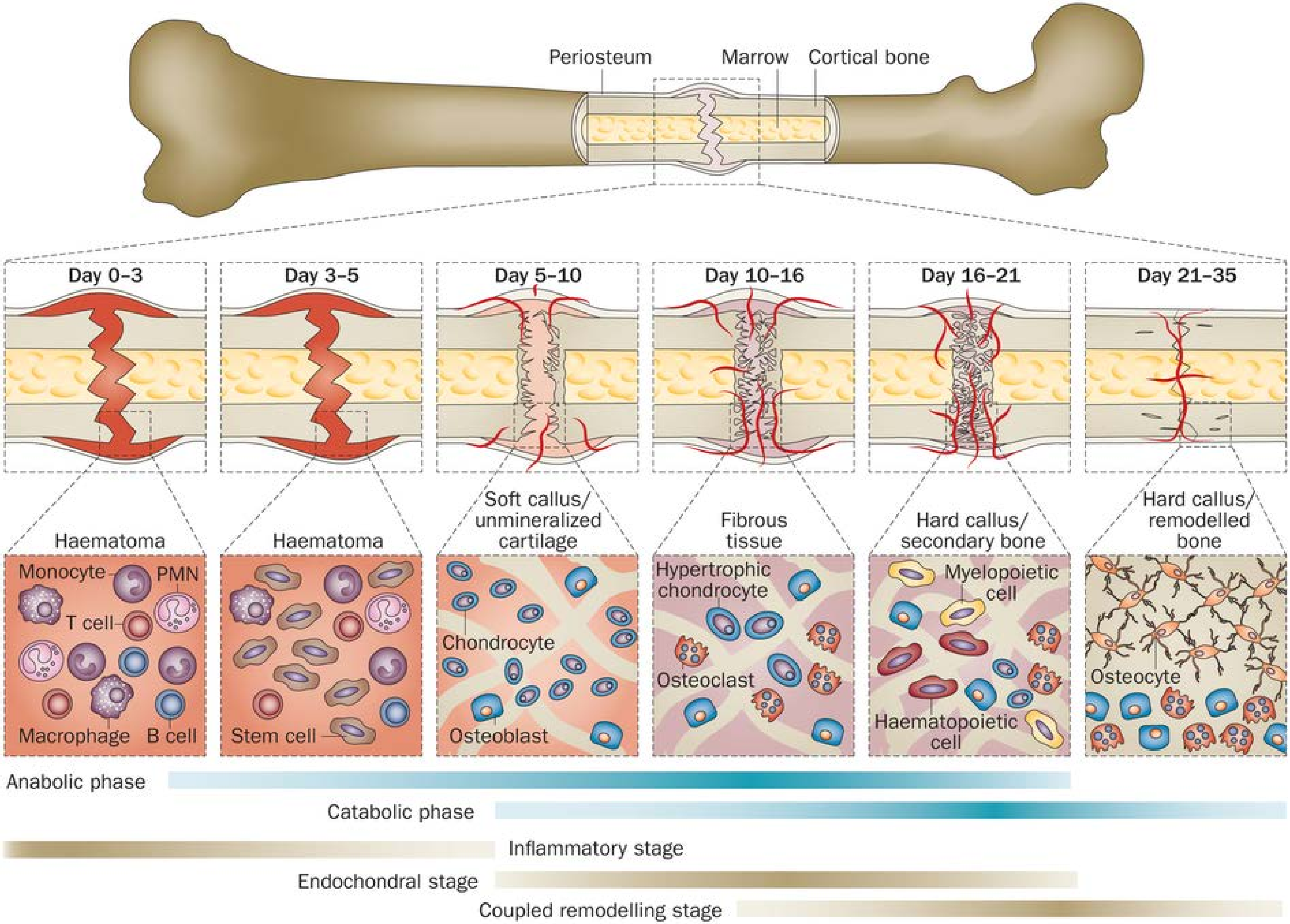
Primary amputation
- Cosmetic/psychological

Distraction Osteogenesis
- Cumbersome
- Infection
- Painful

Autograft- Bone harvested from Iliac Crest
- Painful, expensive, recovery time

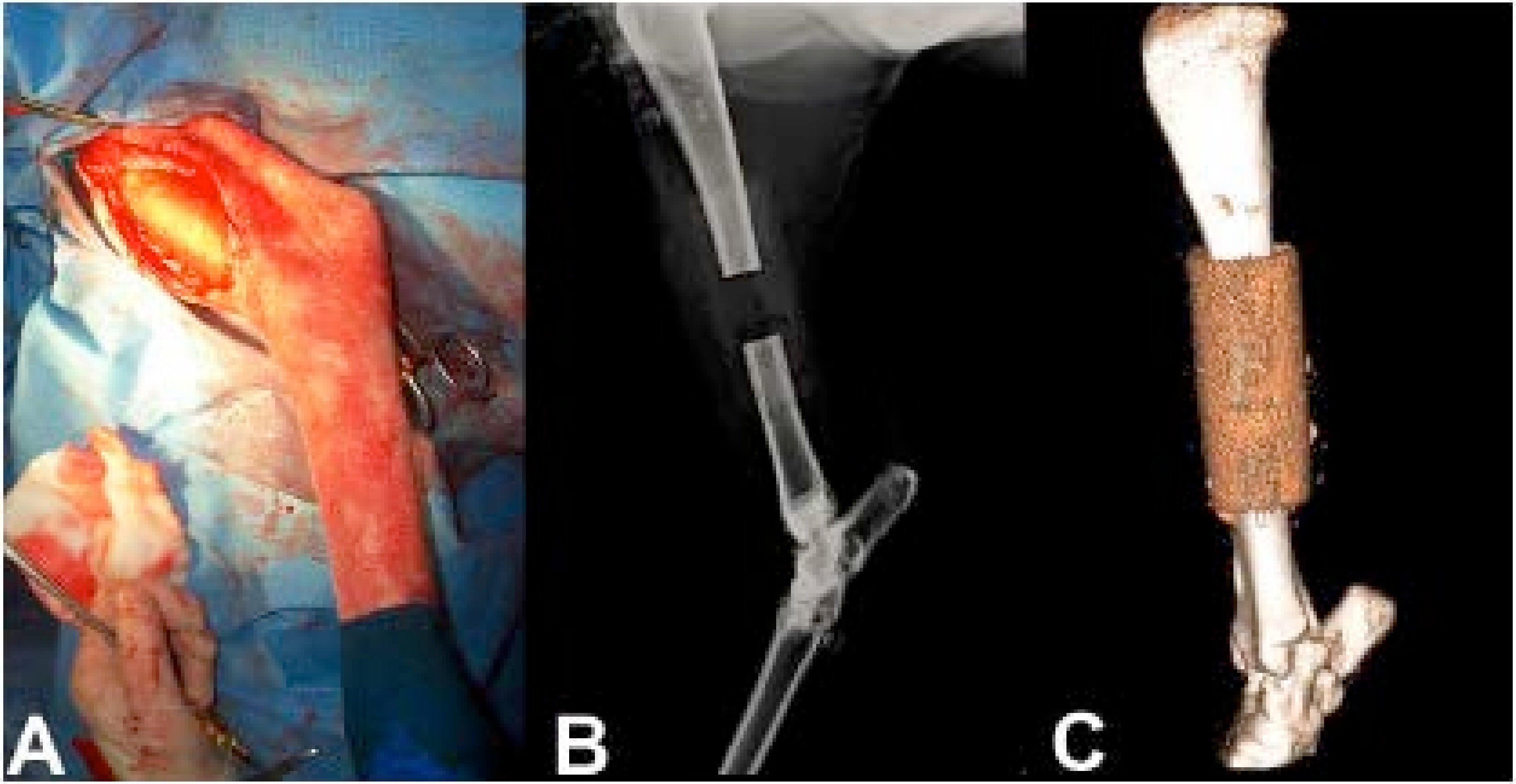
Allograft- Bone harvested from donor
- Possible disease transmission
- Limited availability

Fracture Healing timeline



Nature Reviews Rheumatology 11, 45–54 (2015)

Unmet Medical Need:
Segmental defect following projectile Injury

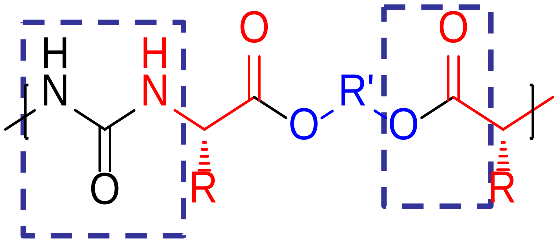


Large Animal Model – Sheep Femur Repair

With Brad K Weiner & Ennio Tasciotti

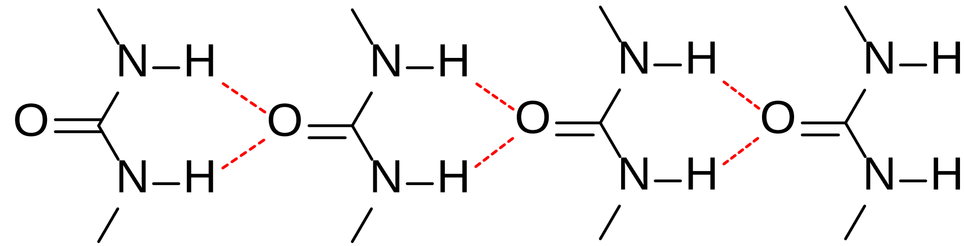
Becker Laboratory for Functional Biomaterials

Amino-acid Based Poly (Ester Urea)s (PEU)



Urea bonds

Ester bonds



H-bonding network

Semicrystalline: Thermal or solution-based processing offers a non-chemical method to tune the mechanical properties, chemical stability and biodegradation rate.

Tunable Degradation:

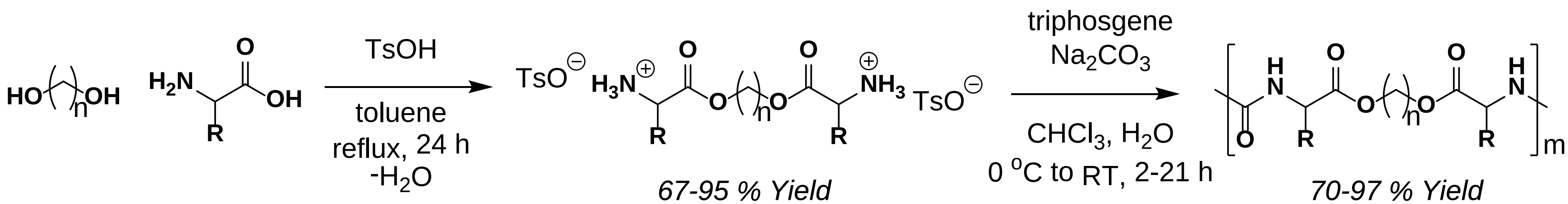
- **Non-toxic byproduct:** Amino acids, CO_2, and readily metabolized diols
- **"Self" neutralization:** No local acidic environment build up and acid-catalyzed degradation

Synthetic flexibility: Many combinations of amino acids and different diols possible

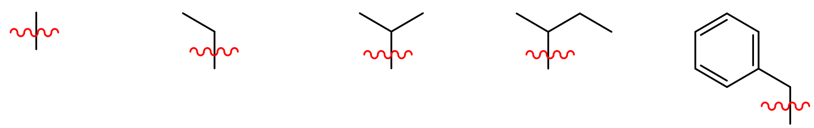
T. Kartvelishvili, G. Tsitlanadze, L. Edilashvili, N Japaridze, R. Katsarava *Macromol. Chem. Phys*., 1997 198,1921-1932.

K Stakleff, F Lin, LA Smith Callahan, MB Wade†, A Esterle, J Miller†, M Graham, ML Becker* *Acta Biomaterialia*, 2013, *9*, 5132-5142.

PEUs have a broad range of properties



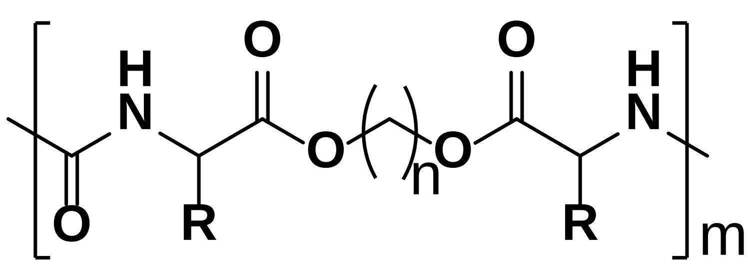
n = 6, 8, 10, 12 **R** = ALA ABA VAL ILE PHE

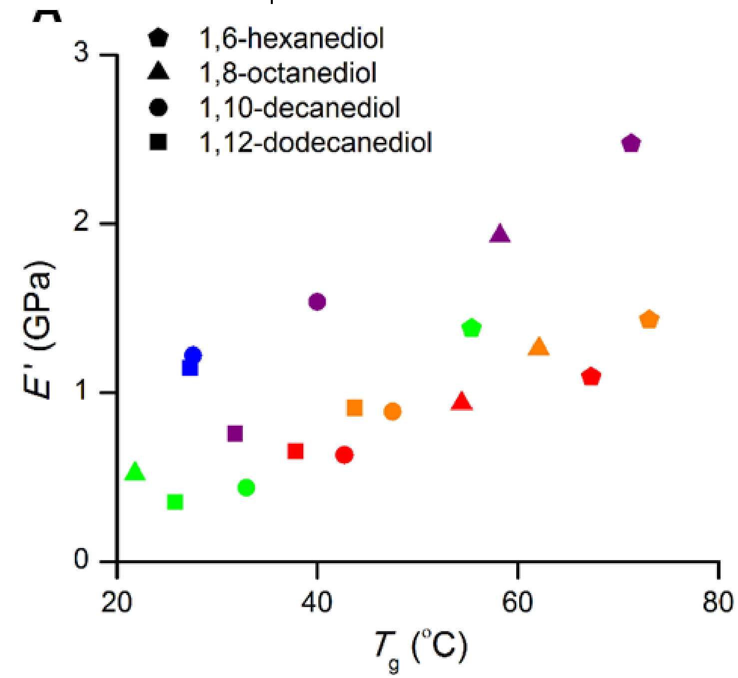


nomenclature:

monomer: m(1-**R-n**) polymer: p(1-**R-n**)

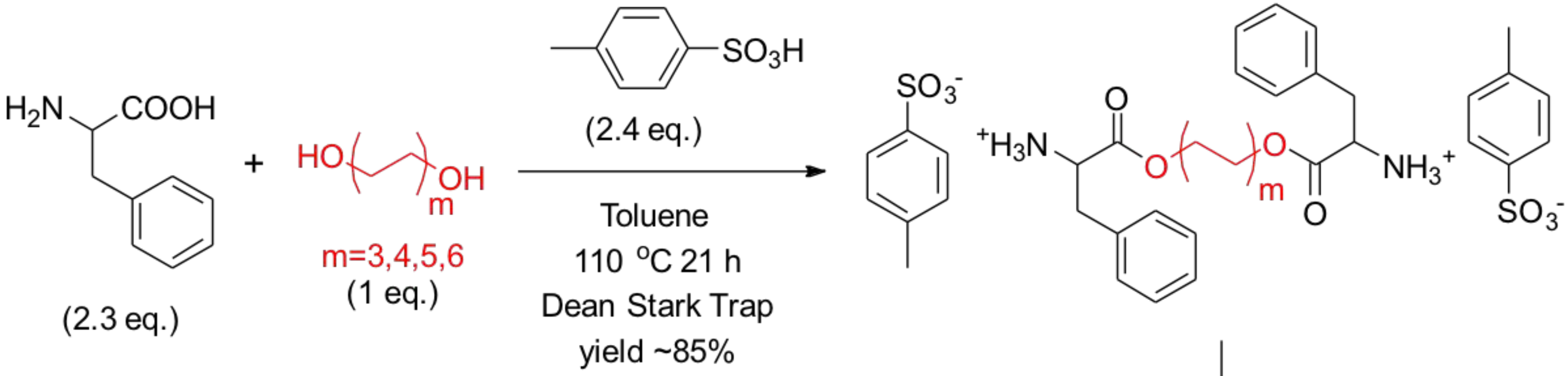
ex. m(1-ALA-12) ex. p(1-PHE-8)

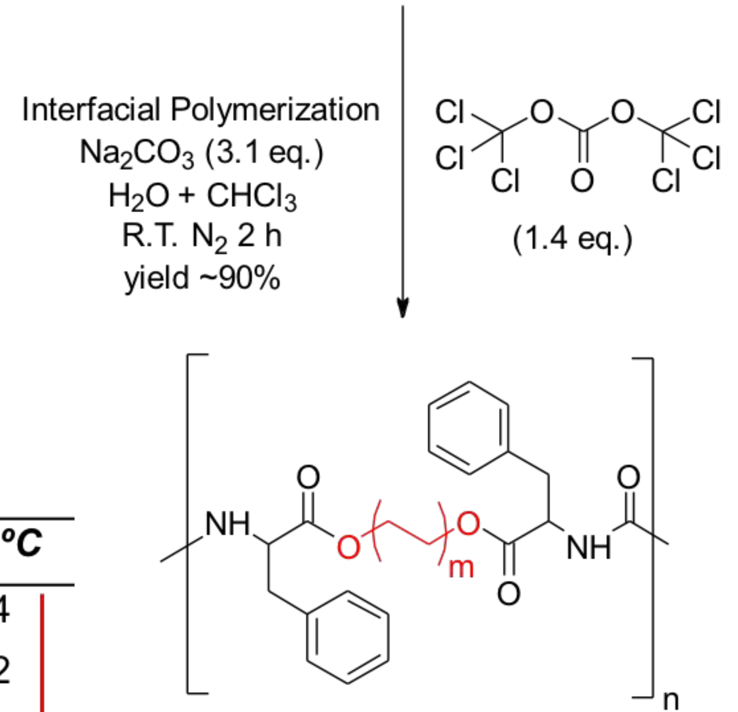




GI Peterson, AV Dobrynin, ML Becker* *ACS Macro Letters*, 2016, *5 (10)*, 1176–1179.
GI Peterson, H Li, EP Childers, AV Dobrynin, ML Becker* *Macromolecules*, 2017, *50 (11)*, 4300–4308.

Synthesis of PEUs with Different Diols



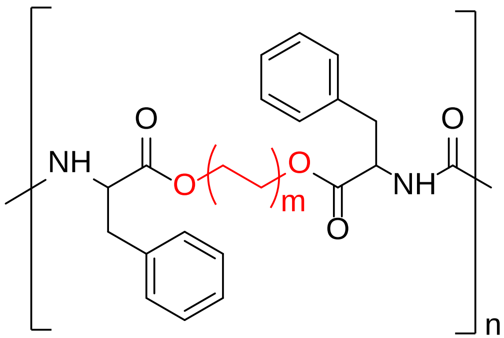


m=3 C6-PHE-PEU
m=4 C8-PHE-PEU
m=5 C10-PHE-PEU
m=6 C12-PHE-PEU

Samples	M_W	$Đ_M$	T_d/ °C	T_g/ °C
C6-PHE-PEU	50 k	1.79	286	54
C8-PHE-PEU	41 k	1.90	287	32
C10-PHE-PEU	63 k	2.11	287	11
C12-PHE-PEU	34 k	2.18	289	3

J Yu, F Lin, Y Gao, P Lin, ML Becker, *Macromolecules*, 2014, 47(1), 121-129.

Tensile Test of PEUs with Different Diol Chain Length



m=3 C6-PHE-PEU
m=4 C8-PHE-PEU
m=5 C10-PHE-PEU
m=6 C12-PHE-PEU

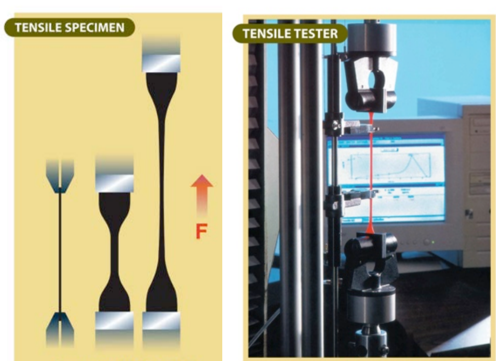


Figure 19: Molded Dumbbell

Figure 20: Stretching a Sample

© 2005 R.L. Hudson & Comp

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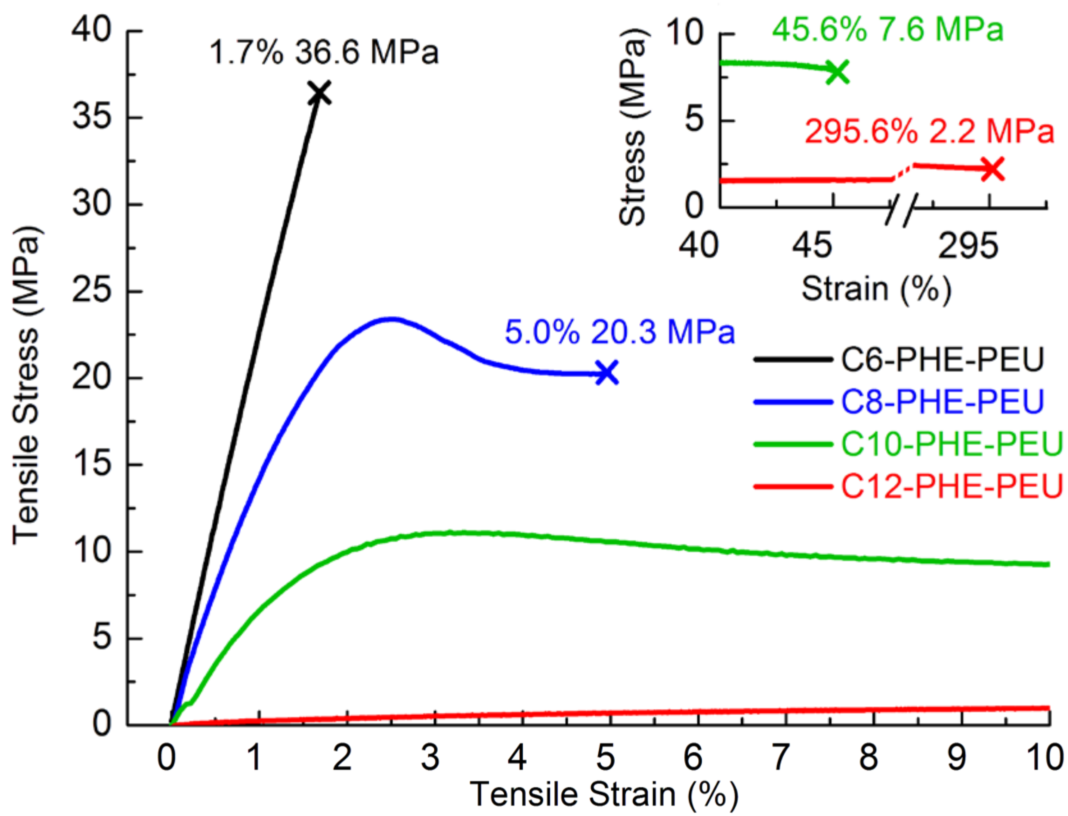
Tensile Test at room temperature



J Yu, F Lin, Y Gao, P Lin, ML Becker, *Macromolecules*, 2014, 47(1), 121-129.

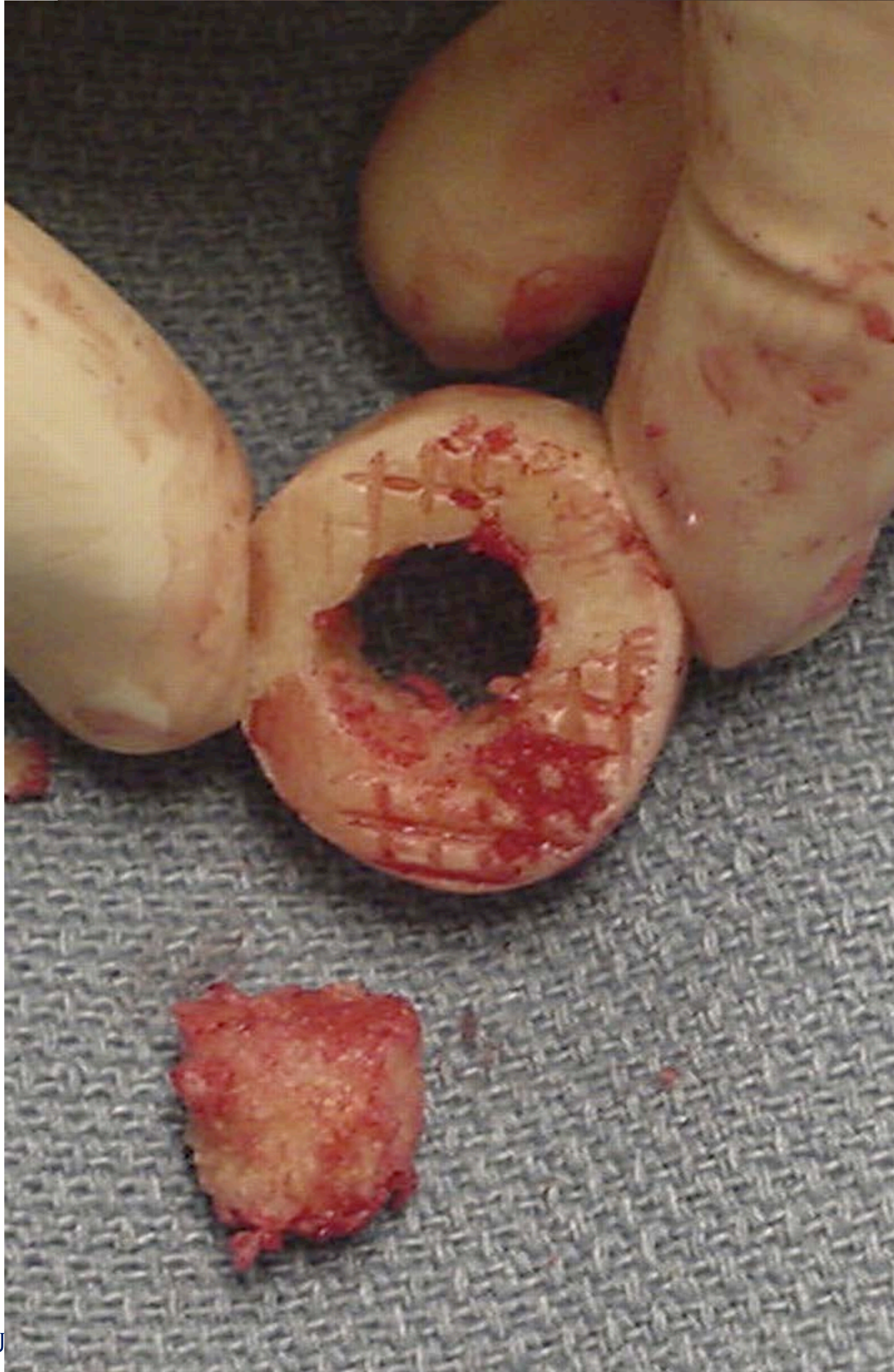
Scaffold for Large Animal Model

Design criteria

1) Single surgery
2) No fixation hardware – glue
3) Tailorable to heights/ bone structure
4) Totally resorbable
5) Maintain structural integrity for 6 months

Orthopaedic surgeons use hammers, saws & chisels… it has to be simple

Collaboration w/
Brad Weiner & Ennio Tascotti – HMRI



Mold Design and Injection Molding

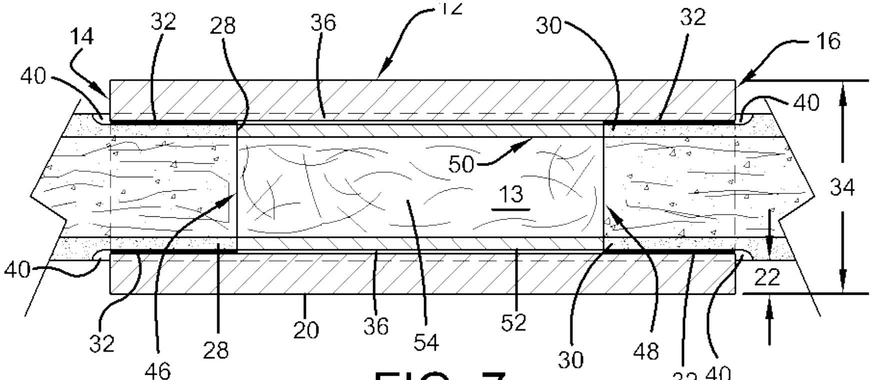
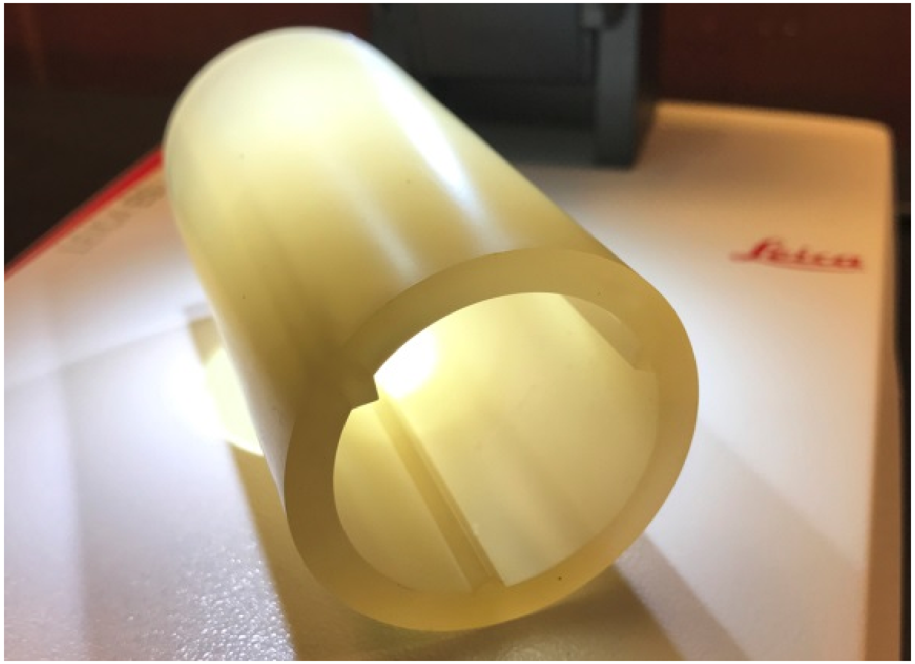


FIG. 7



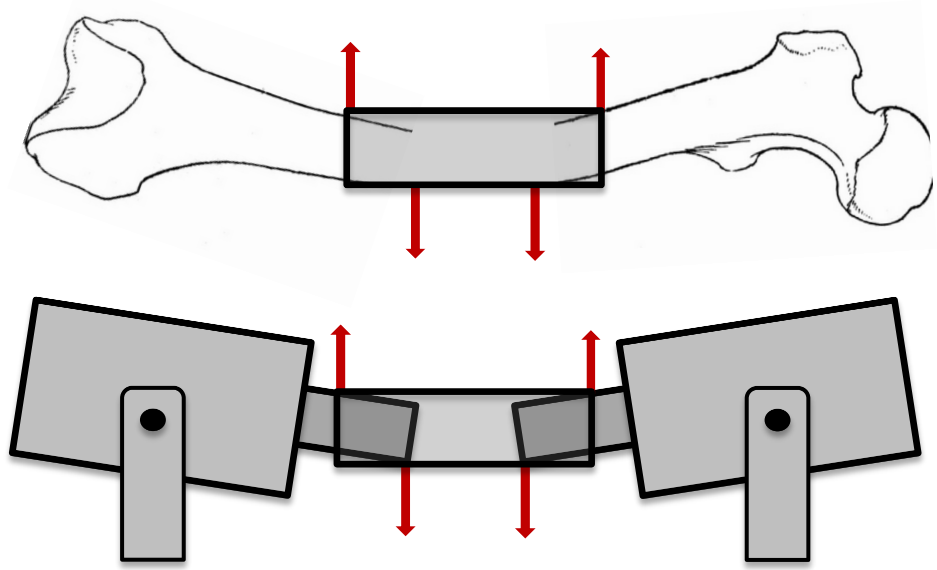
Partnership with Cook Medical

PEU Shells - PCT/US15/30530

Mechanical Strength

w/ Michael Moreno – Texas A&M BME

Hold > 70 kg weight



4 Point Bending Test

Ultimate Force: 1.1 ± 0.2 (10^3N)

Human Femur: 2~5 (10^3N)

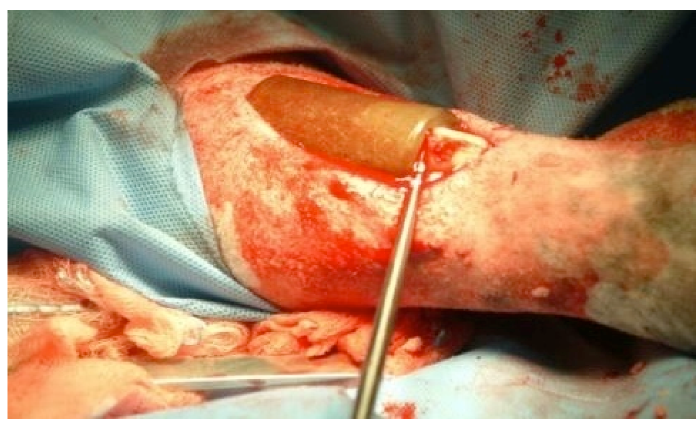
Dog metacarpal: 0.6 (10^3N)

Degradable Poly(ester urea) Shells

Pre –op X Ray

Bone removal

Shell Insertion



Post –op X Ray

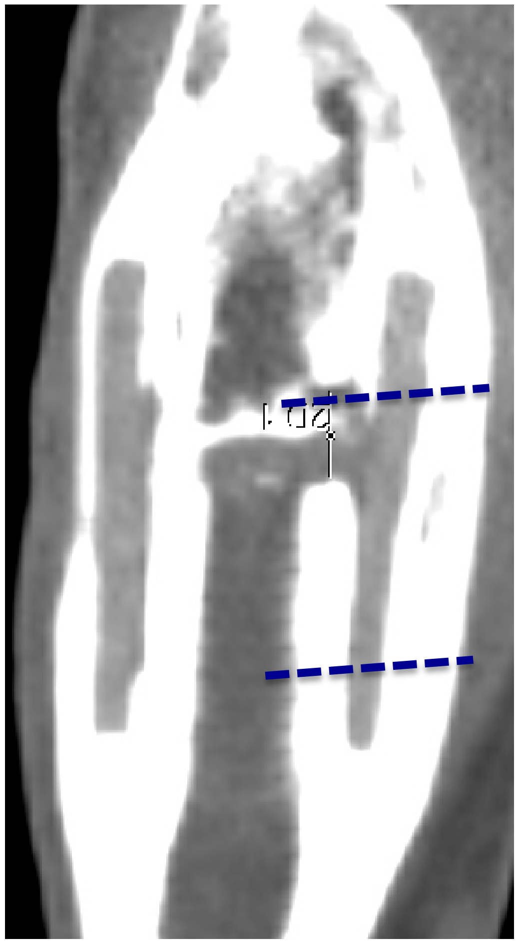
Brad K. Weiner, MD & Ennio Tascotti – Houston Methodist Research Institute

Sheep Tibia X-Rays postop with Control

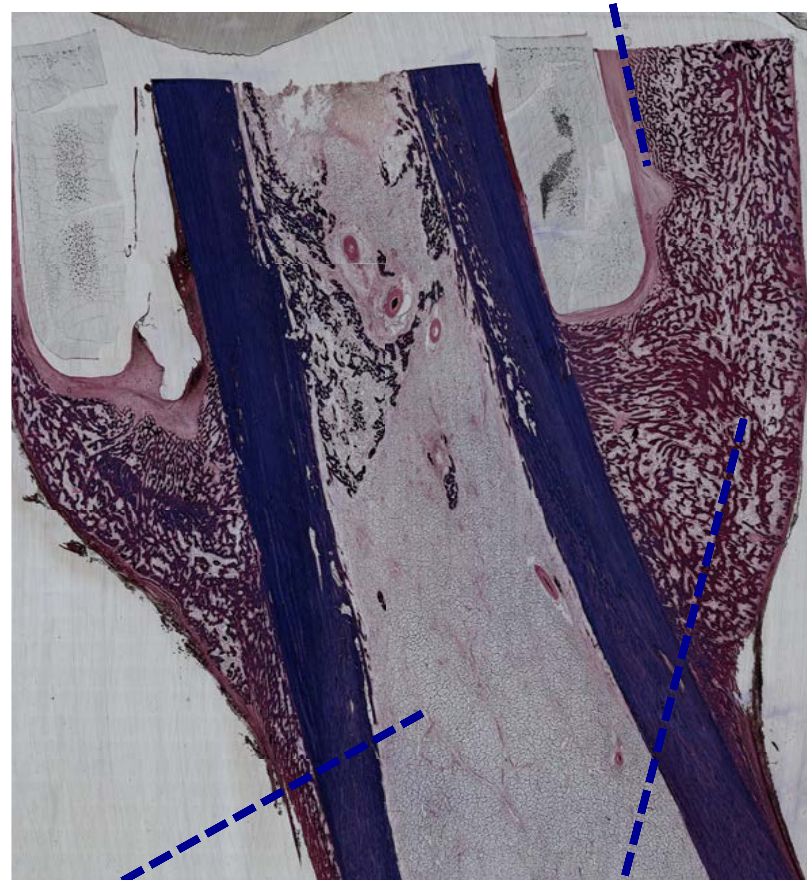
8 hours Post OP

4 weeks Post OP

Histology shows Callus & quality bone formation



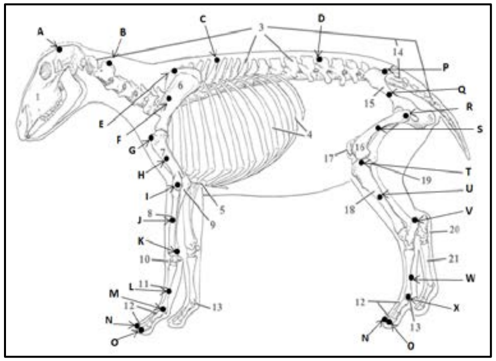
Polymer

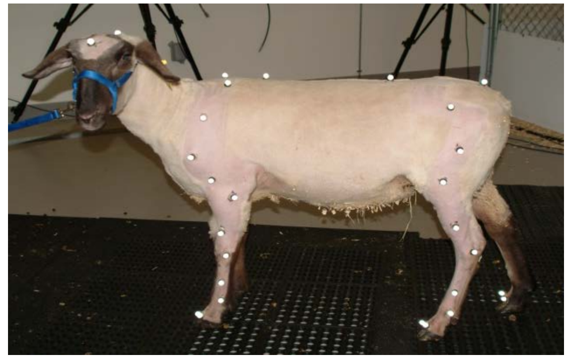


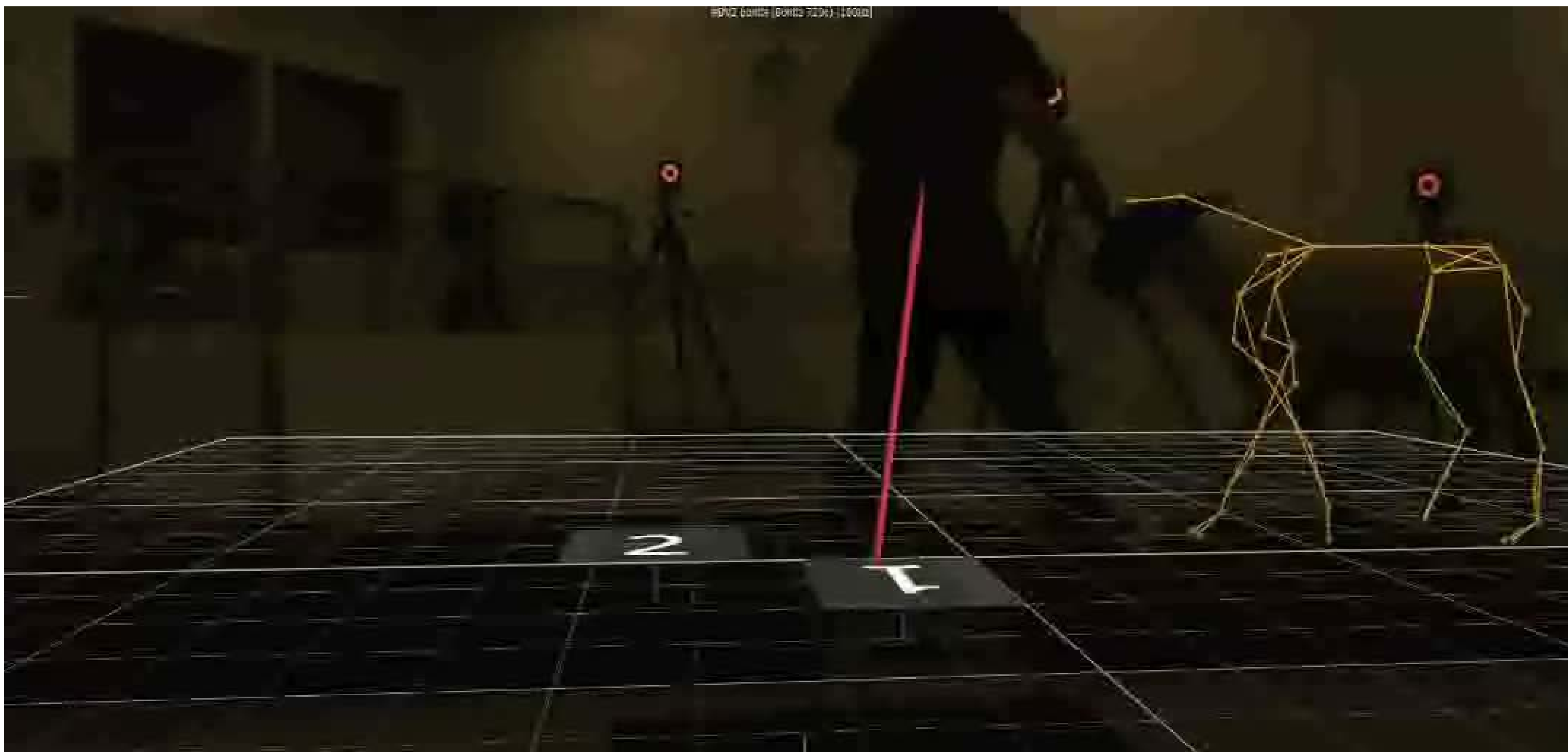
Cortical bone

Callus Formation

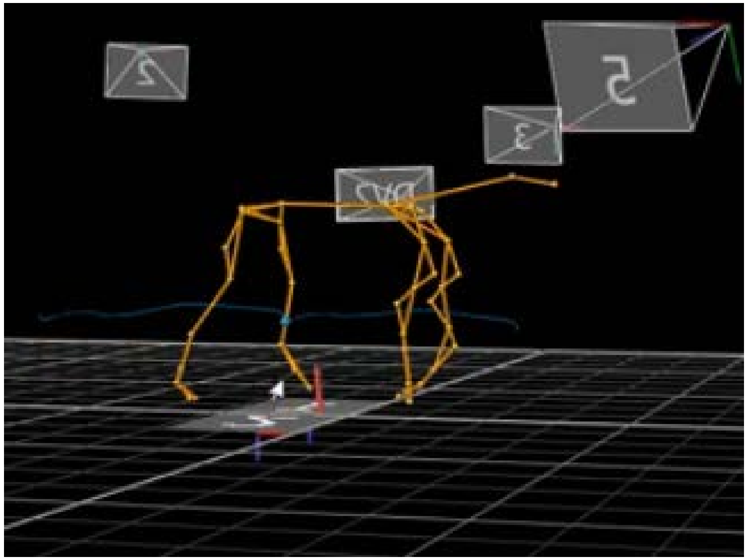
Comprehensive Sheep Dynamics Study to assess functional recovery

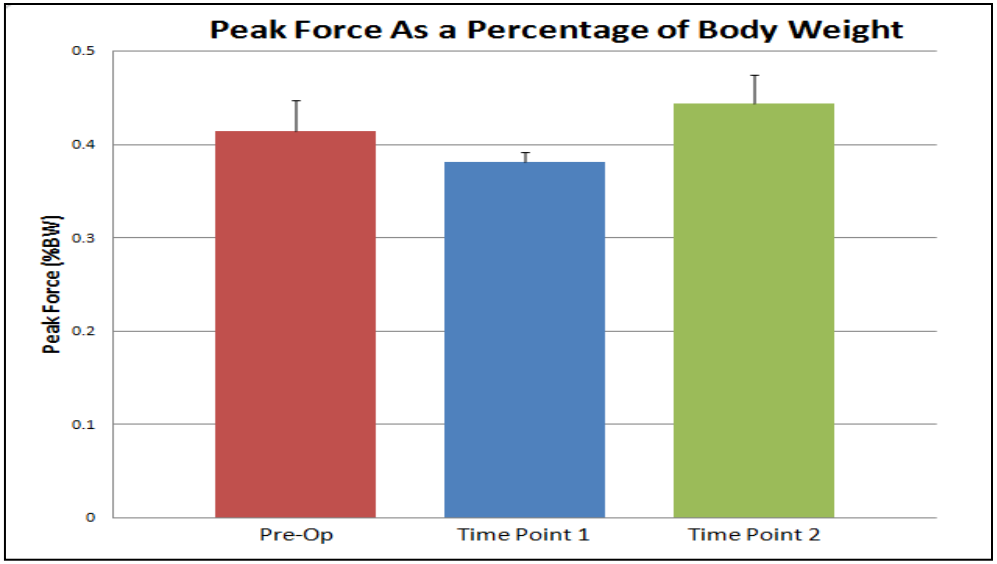


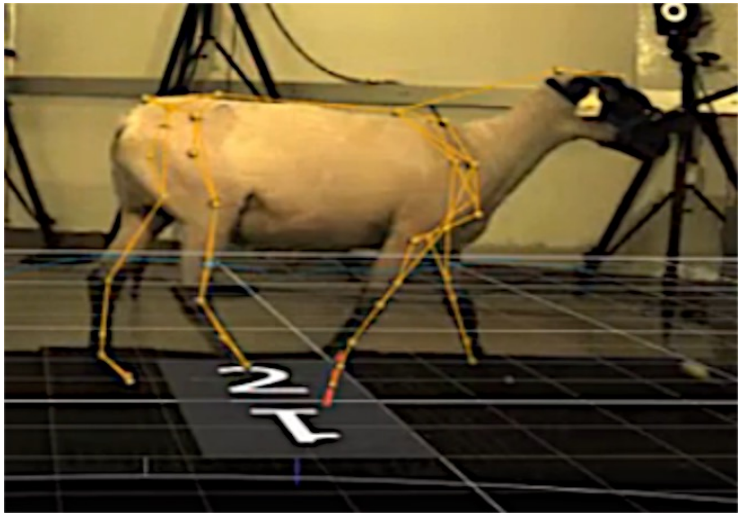


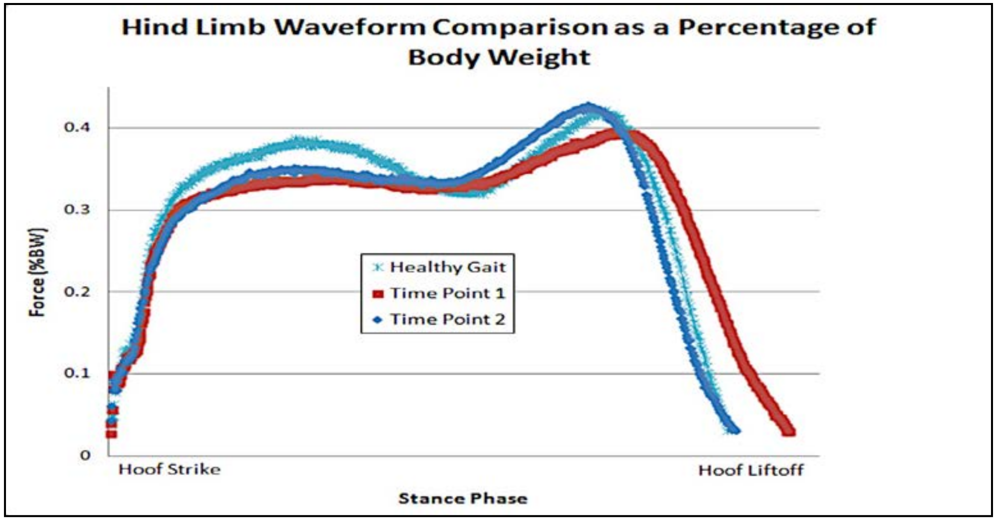


Comprehensive Sheep Dynamics Study to Assess Functional Recovery

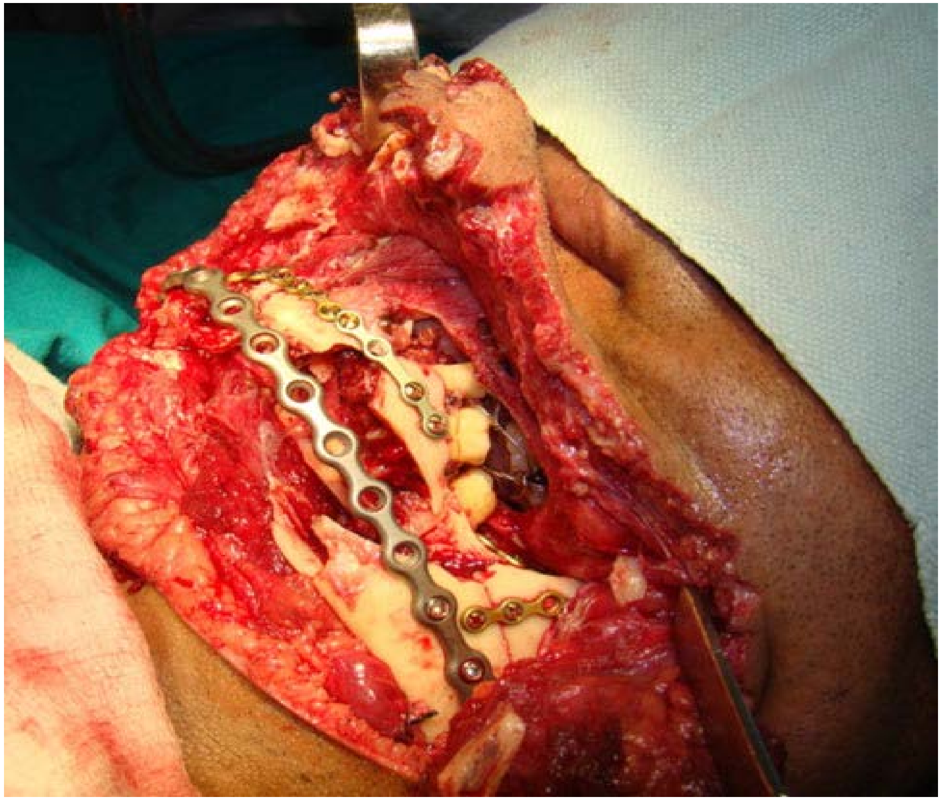








The Future: Patient Specific – Anatomically Correct



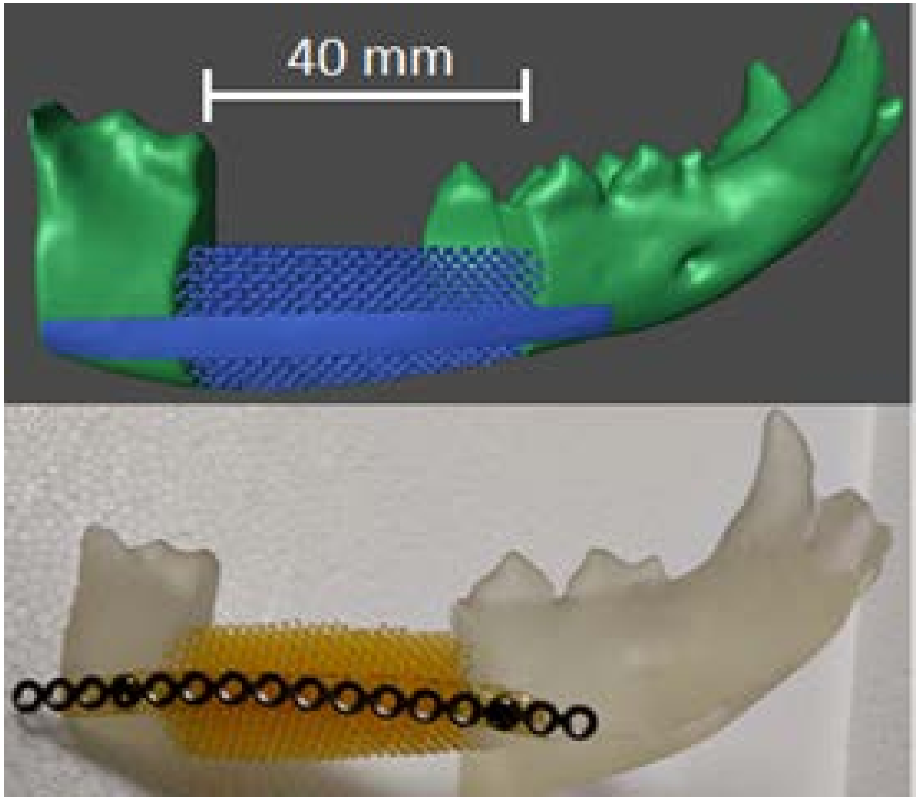


Image → Digital Design → Conversion to Print Code → Printing

Only "works" when the optimal materials are available

Pre-operative 3D Printing of Neurocranial Deficit Implants

Preoperative Fabrication Saves OR Time **($150-300/minute) in Akron**

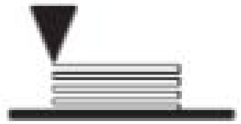
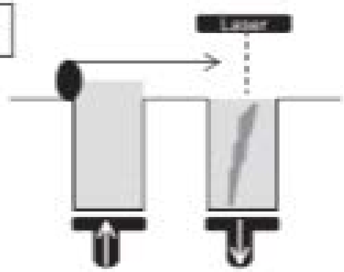
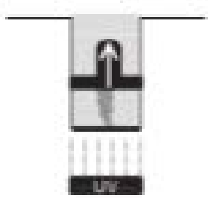
Skull model (beige),

Surrogate (red),

Implant (white).

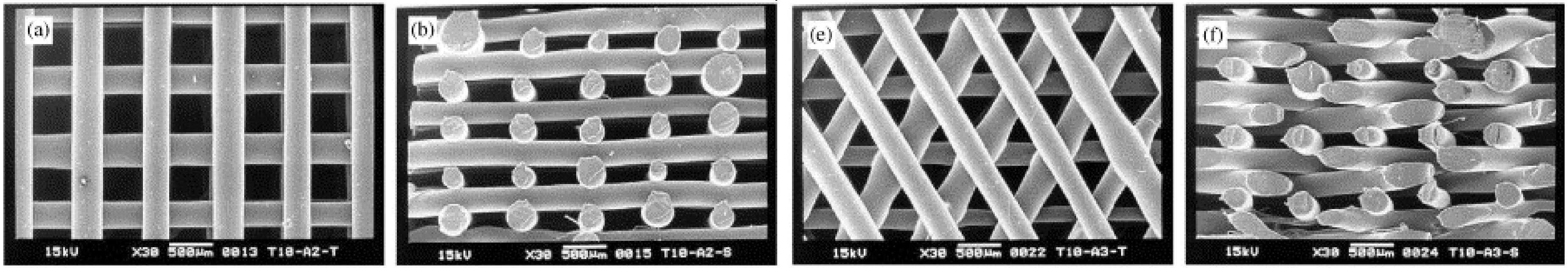
Dean D, Min K-J, Bond A: Computer Aided Design of Pre-fabricated Cranial Plates. J. Craniofacial Surgery, **2003**, 14:819-832,.

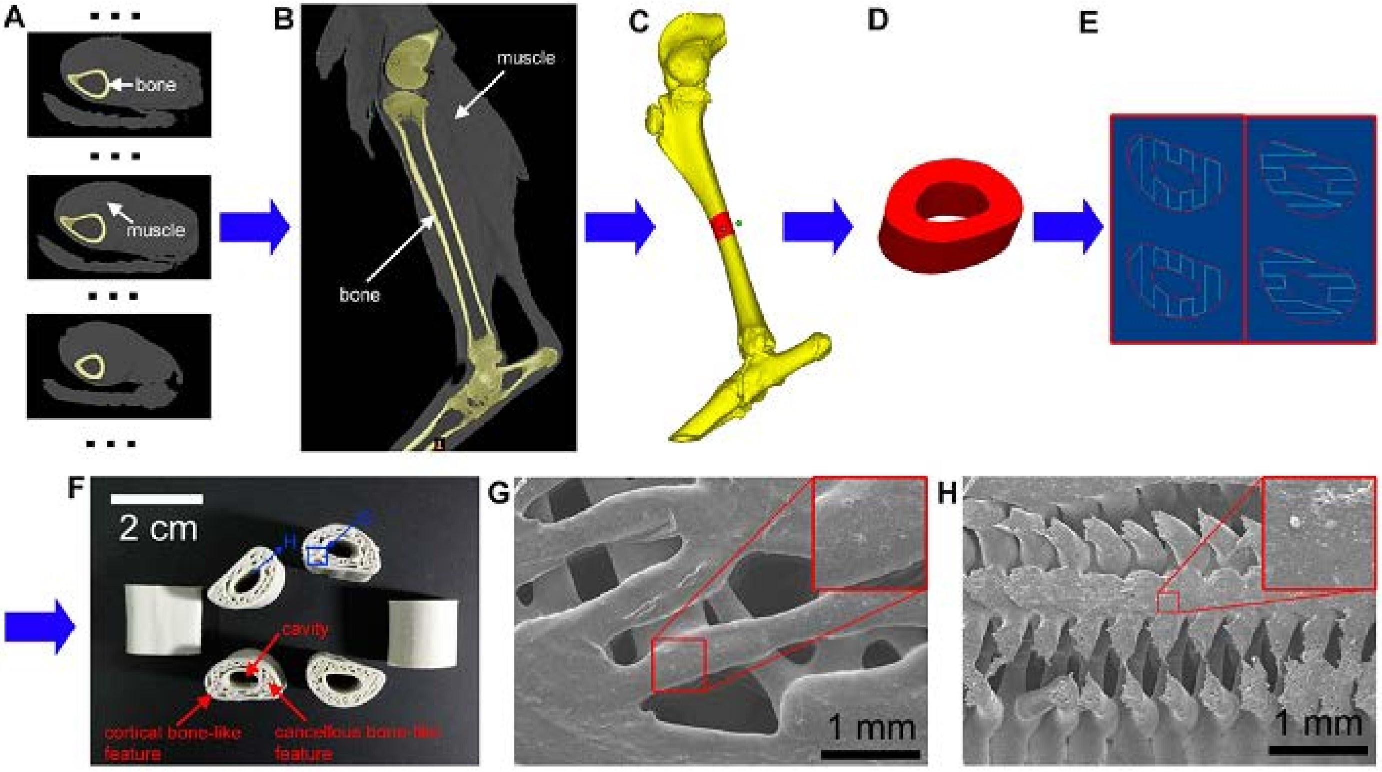
3D Printing is not "New"

Types	Description	Materials
a 	– Melt extrusion – Through a heated nozzle, polymer or ceramic is extruded and printed layer by layer in the *x* and *y* plane, while a stage moves in the *z* plane.	– Poly(lactic acid) (PLA) – Acrylonitrile butadiene styrene – Poly(vinyl alcohol) – Poly(caprolactone) – Nylon – Poly(carbonate)
b 	– Powder and binder deposition – A polymer solution is extruded through a nozzle in a layer-by-layer fashion. – Prints with milder conditions to facilitate printing of biomolecules, proteins, and cells	– Poly(caprolactone) – PLA/poly(ethylene glycol) – Hydroxyapatite – Bioactive glasses
c 	– Powder sintering – A layer-by-layer polymer powder addition is fused/printed by sintering each layer with a laser. – Full melting, partial melting, and liquid phase sintering	– Nylons – Poly(styrene) – Steel – Titanium – Composites – Green sand – Poly(carbonate) – Wax
d 	– Photo-polymerization – A moving platfrom allows a polymer solution to flow on top of the previous layer. Through ultraviolet treatment, the solution is solidified from the patterning of the light source.	– Poly(propylene fumarate) – Poly(acryiate) – Trimethylene carbonate – ε-caprolactone – D,L-lactide

EP Childers, M Wang, JP Fisher, ML Becker, D Dean* *MRS Bulletin* **2015**, *40(2),* 1119-1126.

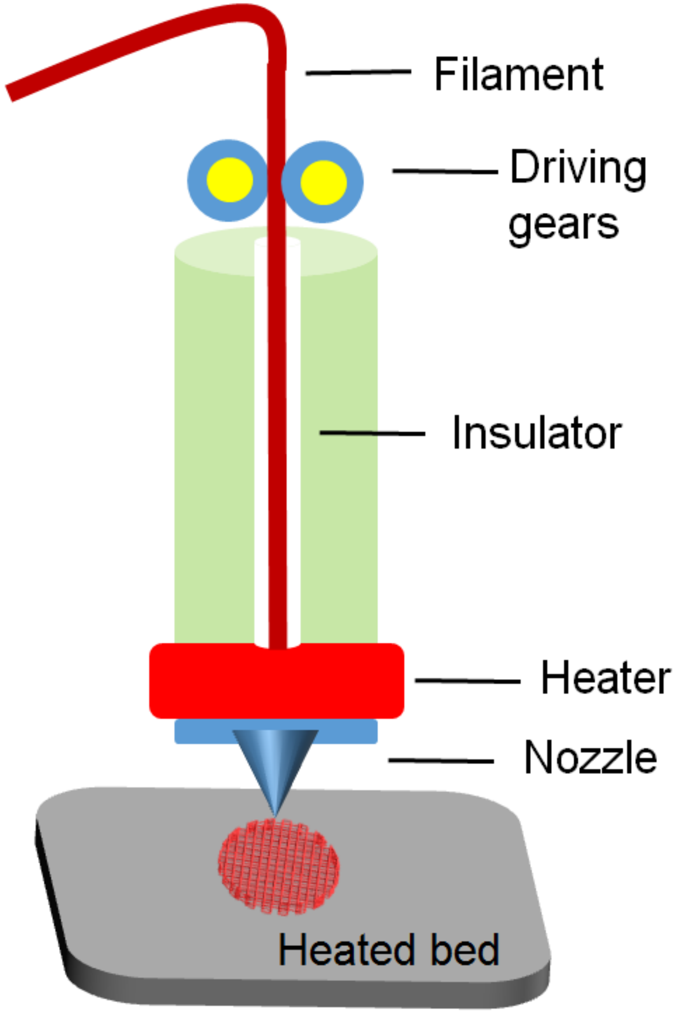
FDM 3D Printing

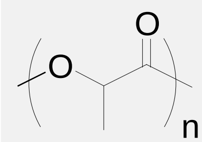
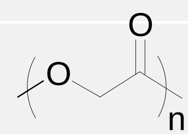
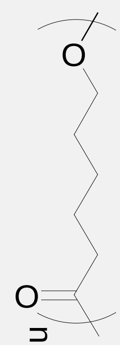




N Xu, X Ye, D Wei, J Zhong, Y Chen, G Xu, D He. *ACS Applied Materials & Interfaces*. 2014, 6, 14952-14963.

Biologics Are Deactivated During FDM Printing

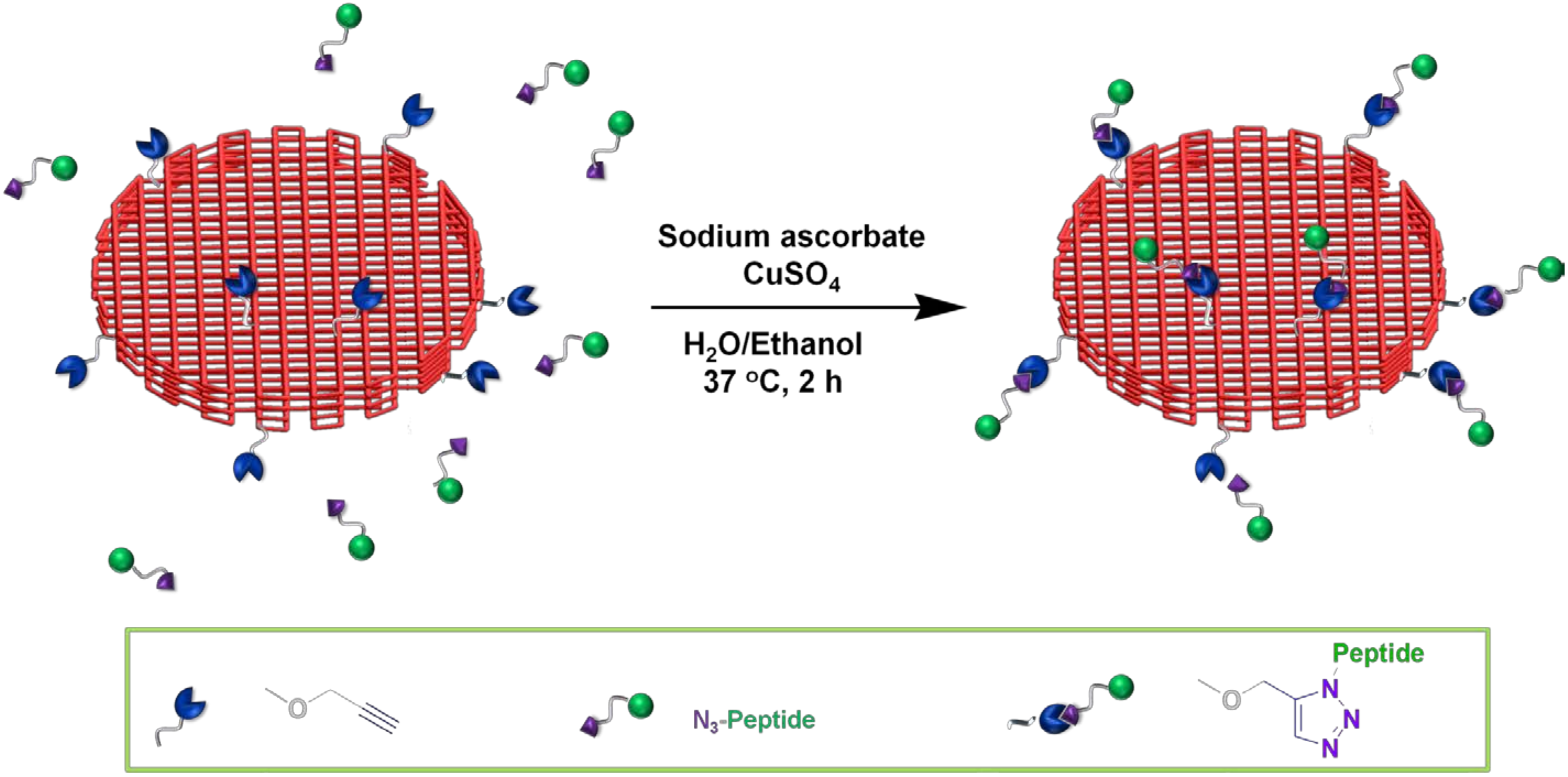


	Chemical structure	T_g (C)	T_m (C)
PLLA		60-65	173-178
PGA		35-40	225-230
PCL		-65-60	58-63
		too high for most biologics to survive	

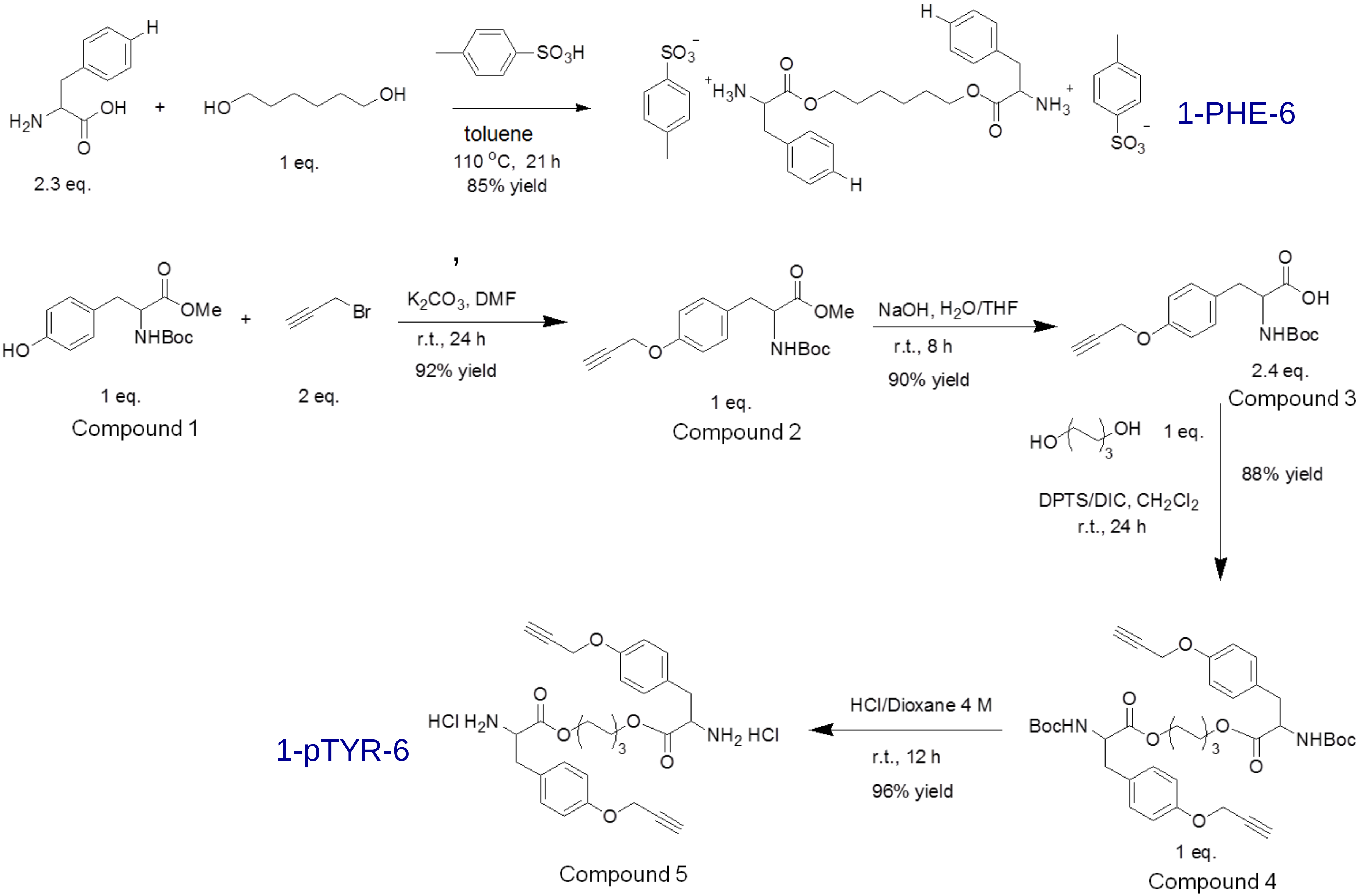
Post-printing surface functionalization is needed.

1. PA Gunatillake, R Adhikari. *Eur. Cell Mater*. 2003, *5*, 1- 16.
2. MI Sabir, X Xu, L Li. *J. Mater. Sci.* 2009, *44*, 5713-5724.
3. S Wang, L Lu, MJ Yaszemski. *Biomacromolecules*. 2006, *7*, 1976-1982.

How do we incorporate biologics ? Contrast?

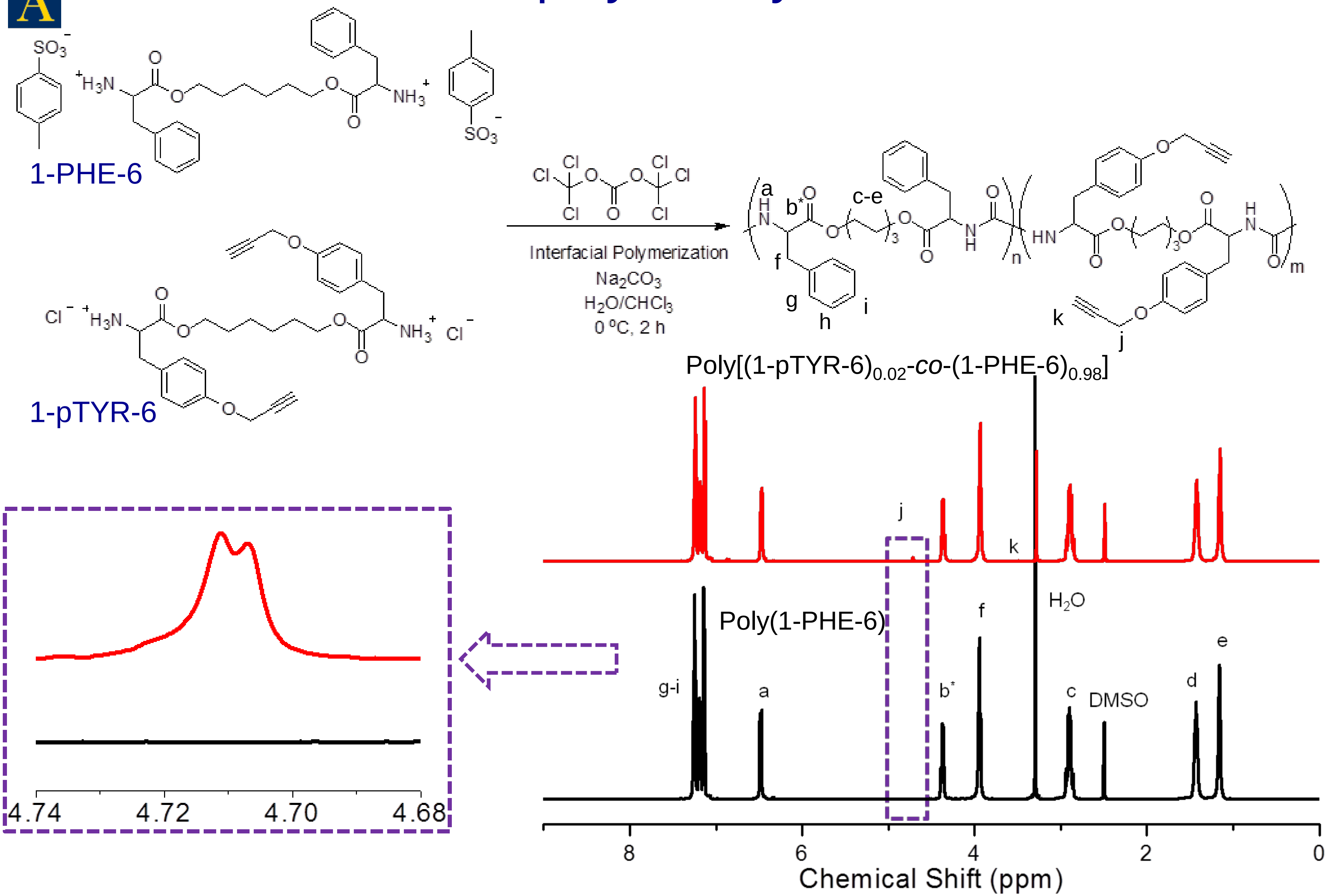


Monomer Synthesis

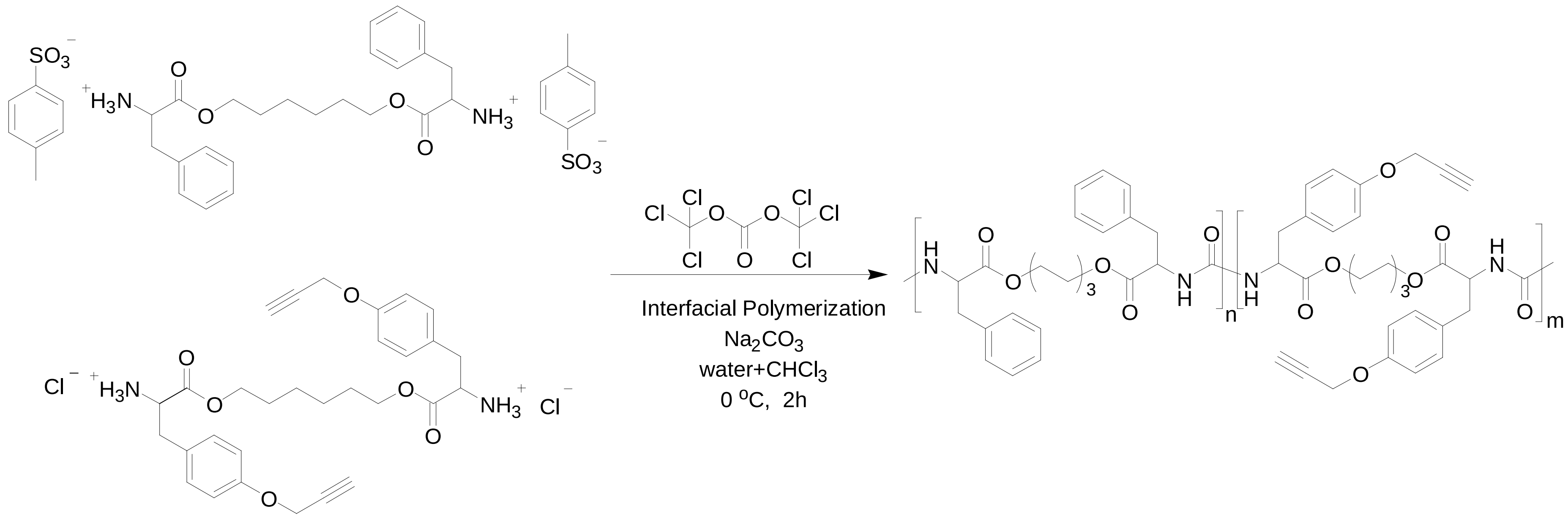


Becker Laboratory for Functional Biomaterials

PEU Copolymer Synthesis

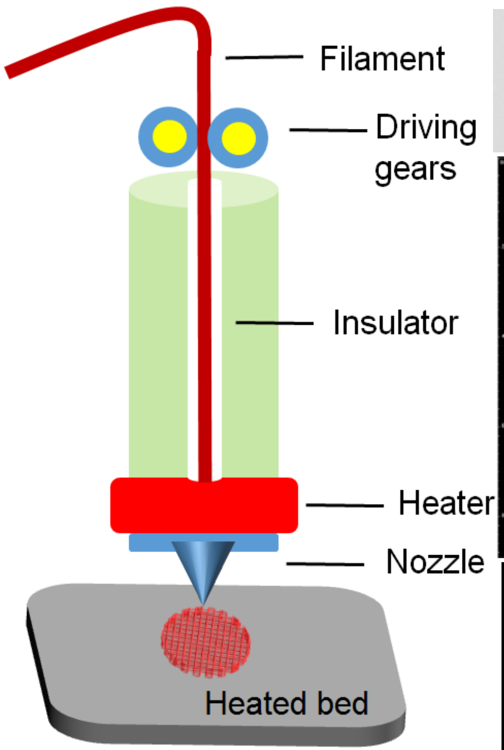


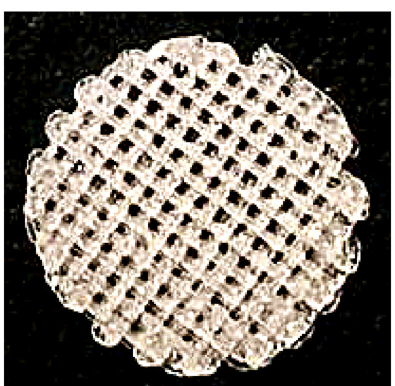
PEUs Synthesis & Characterization

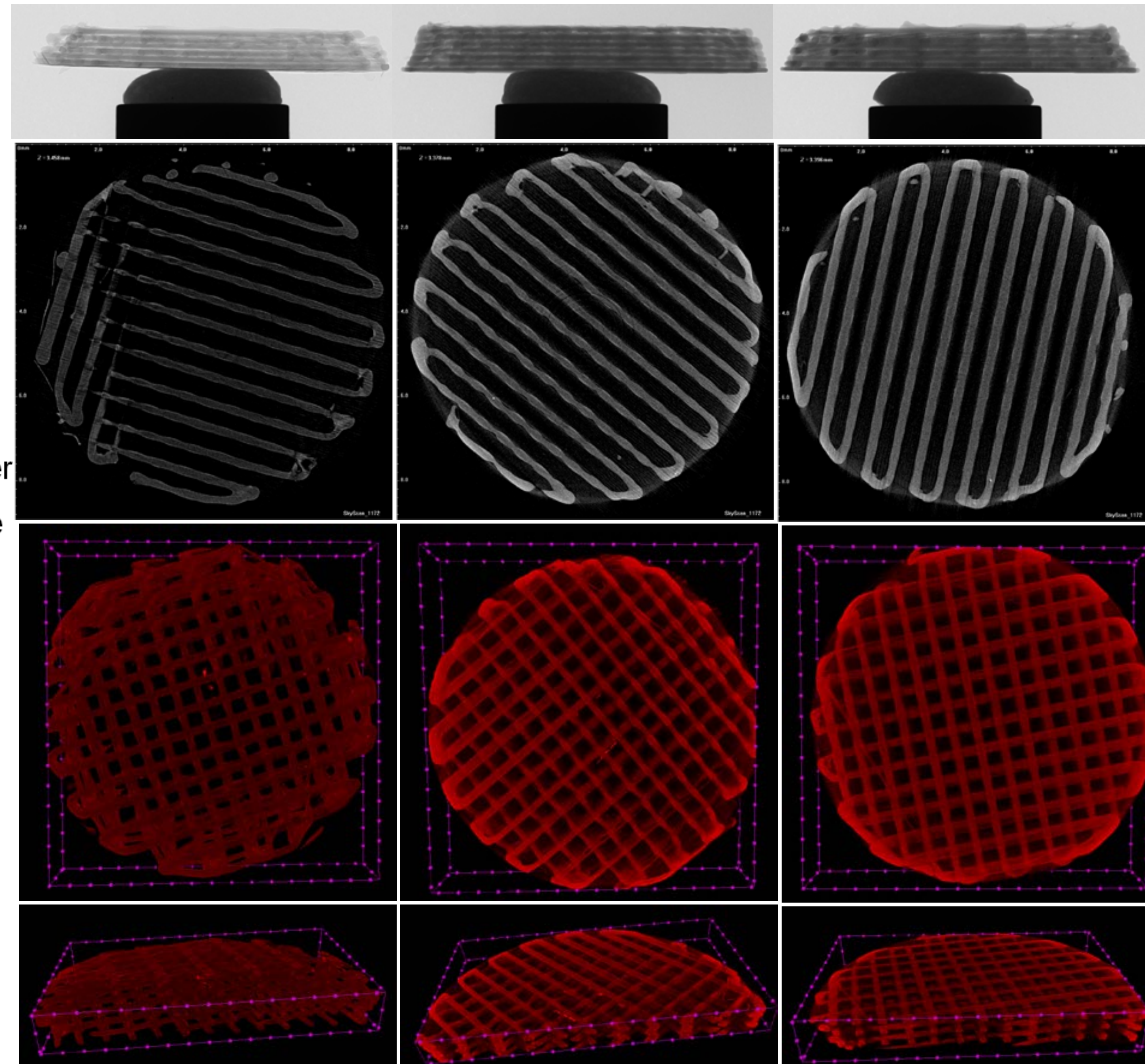


Sample	PEU Composition	M_w Polymer	Filament	Scaffold	$Đ_M$ Polymer	T_d/°C	T_g/°C
Poly(1-PHE-6)	0:100	134k	105k	84k	1.7	294	59
Poly[(1-pTYR-6)$_{0.02}$-*co*-(1-PHE-6)$_{0.98}$]	2:98	108k	92k	85k	1.6	299	59

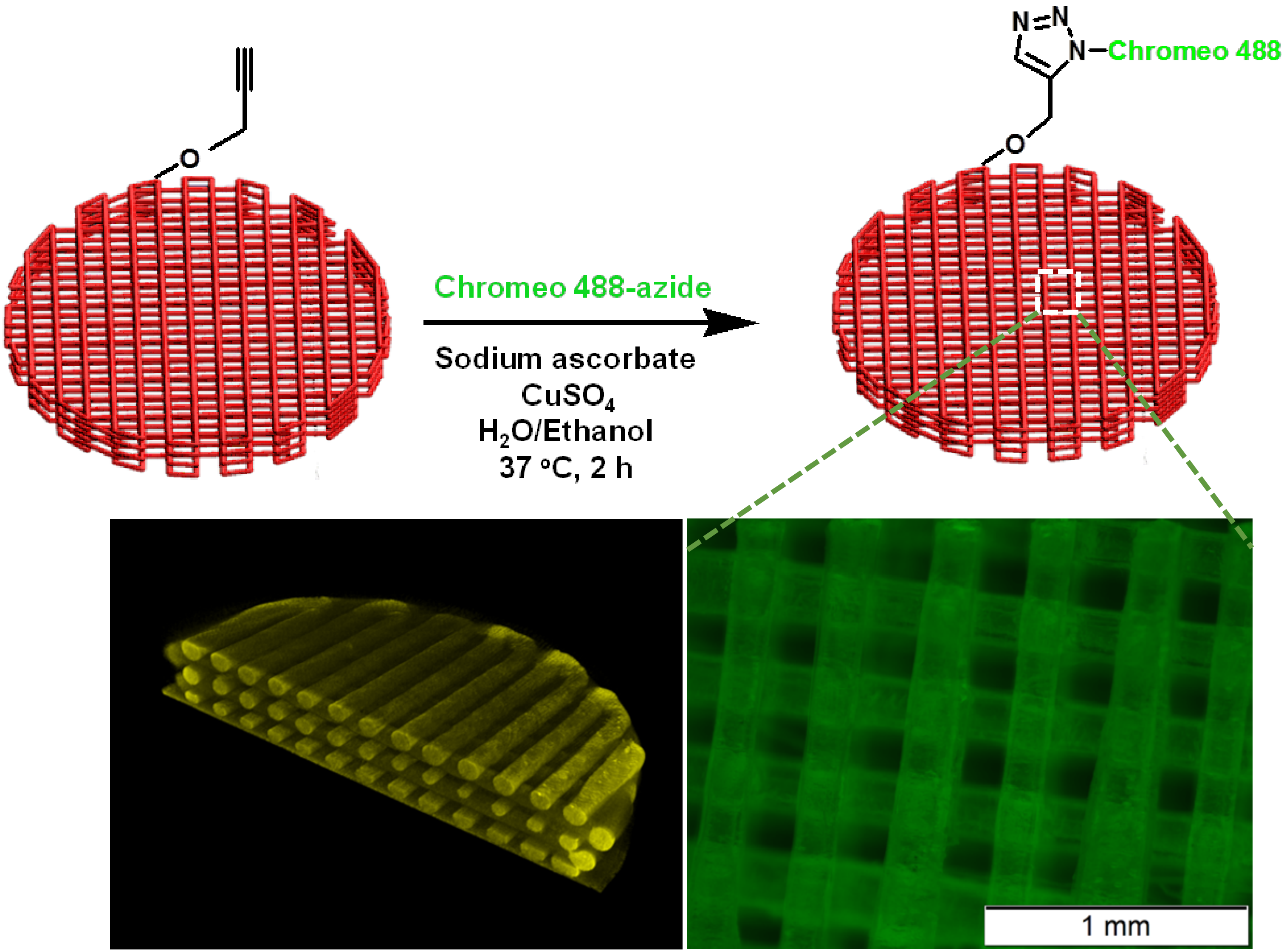
FDM Printing – 200 um struts, 400 um pores





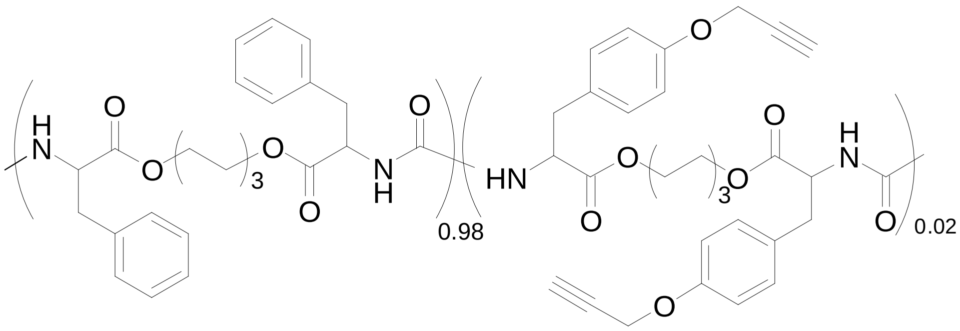


Chromeo488-azide Attachment to Scaffold



Diameter: 8 mm; thickness: 1 mm; layer height: 150 µm; fill density: 20%.
Strut size: 200 µm; pore size: 400 µm.

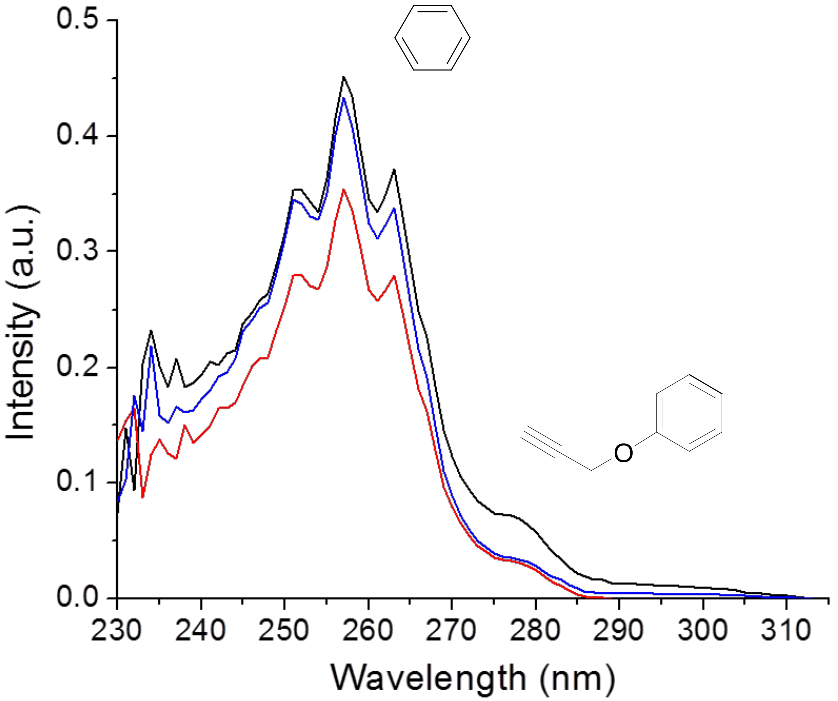
Concentrations via UV-Visible Spectroscopy

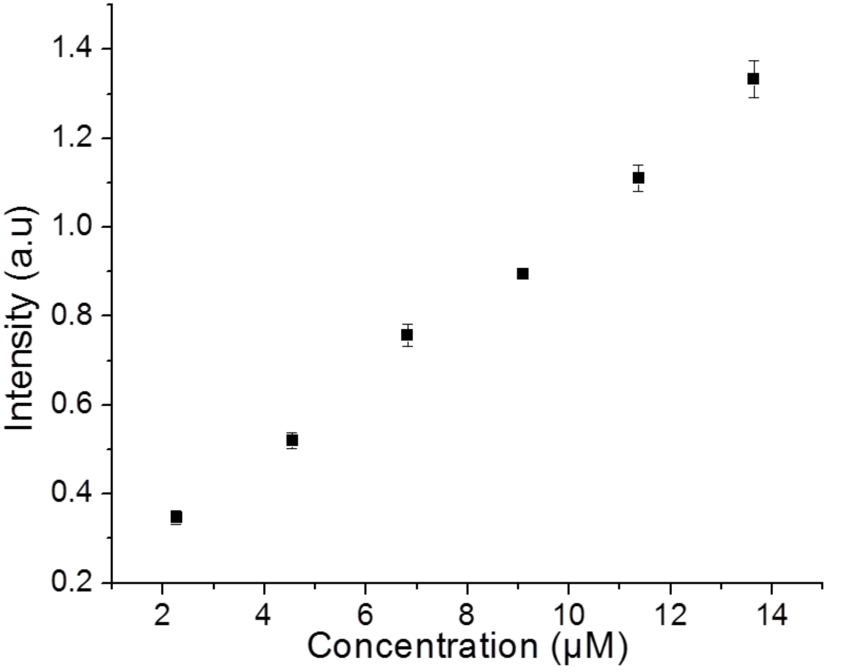


Black curve: PEU + dye without Cu catalyst;

Red curve: PEU + dye with Cu catalyst.

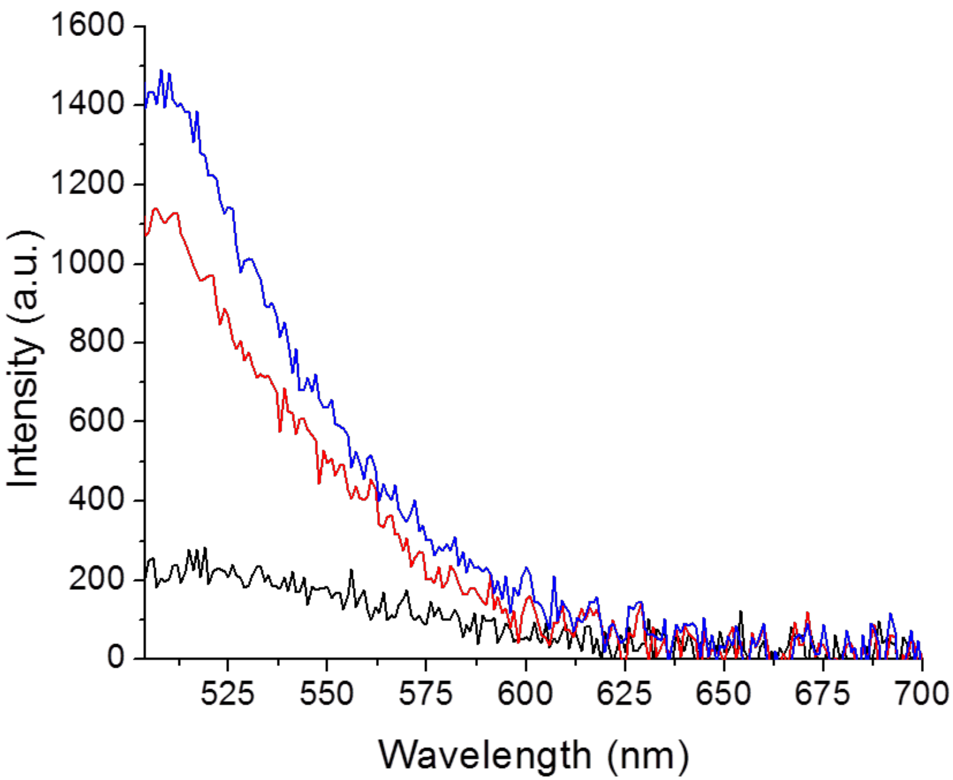
~75 pmol/cm^2

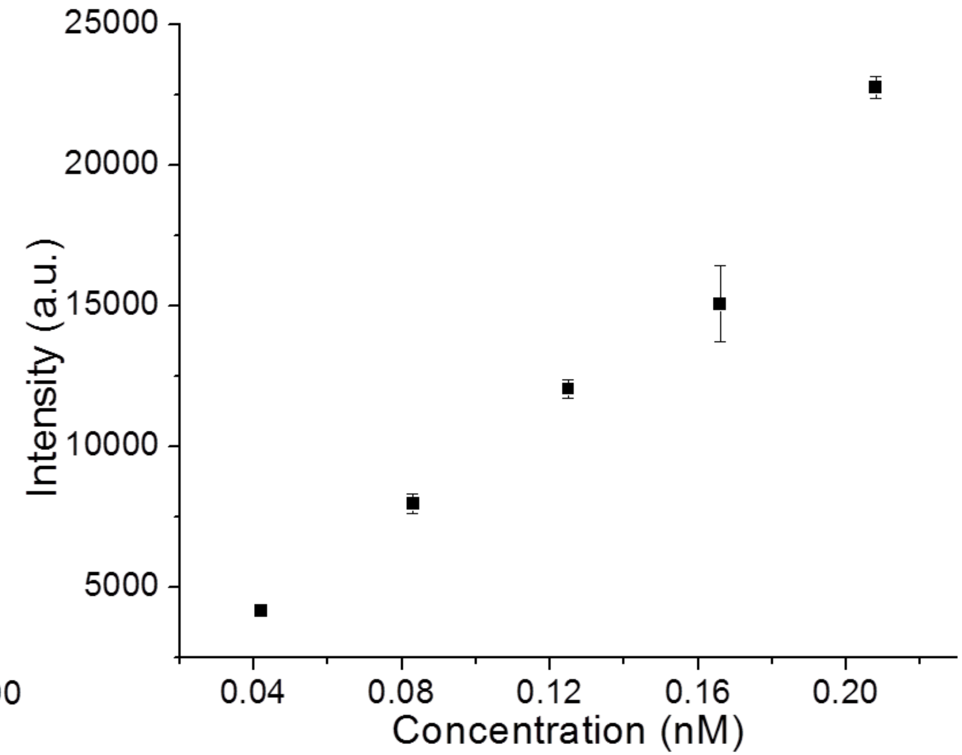




Concentration Determination via Fluorescence

Black curve: Functionalized PEU (after EtO) + dye without Cu catalyst;
Blue curve: Functionalized PEU (after EtO) + dye with Cu catalyst;
Red curve: Functionalized PEU (before EtO) + dye with Cu catalyst.





The surface functional group density is (75 5) pmol/cm^2.
Functional group remains unaffected after EtO sterilization;

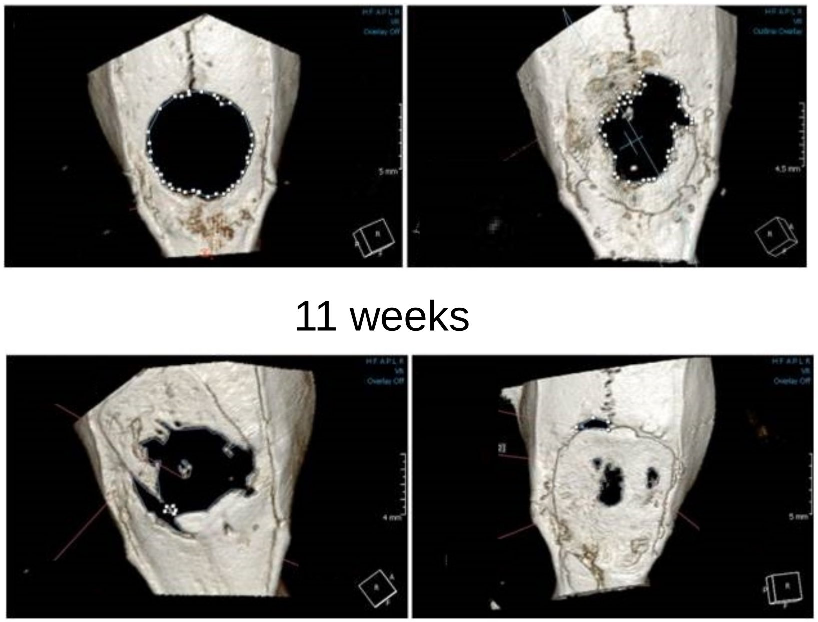
Biologics into/ on Polymers Enhance Function

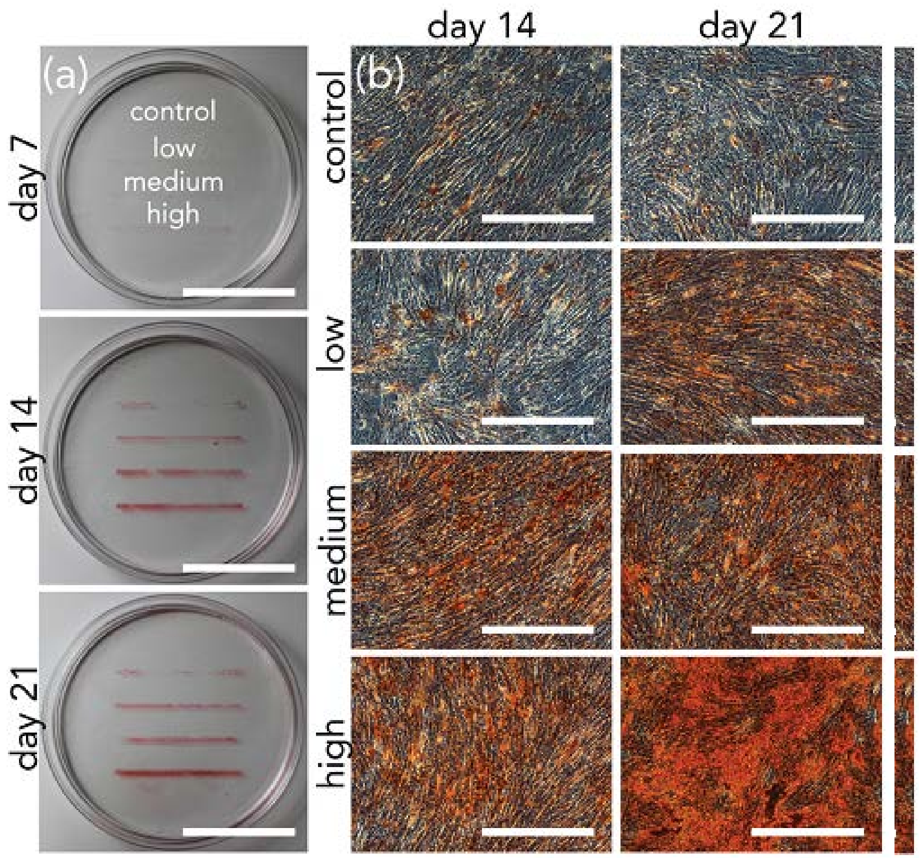
Rat cranial defect recovery

BMP-2 unloaded scaffold   BMP-2 loaded scaffold

4 weeks

11 weeks



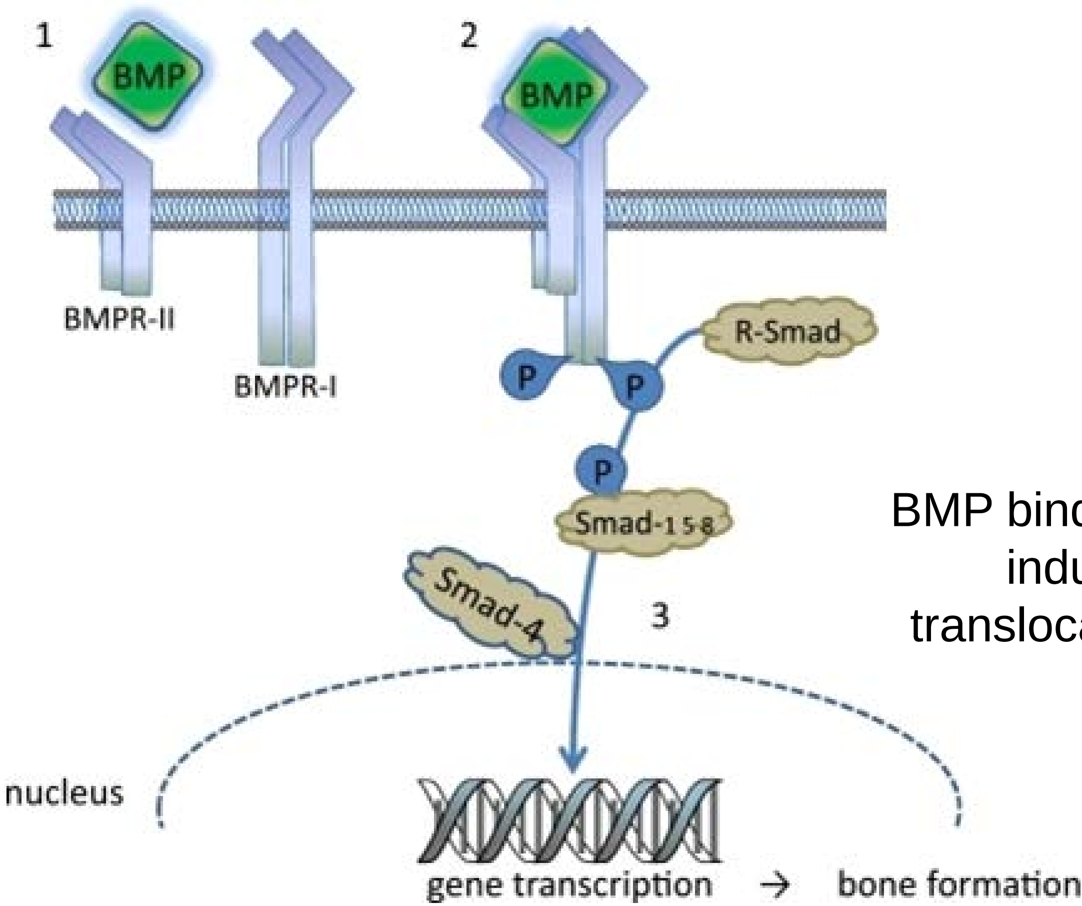


The bioactivity of biologics is concentration dependant.

JW Lee, KS Kang, SH Lee, JY Kim, BK Lee, DW Cho. *Biomaterials*, 2011,32, 744-752.
H Tao, B Marelli, M Yang, B An, MS Onses, JA Rogers, DL Kaplan, FG Omenetto. *Advanced Materials*, 2015, 27, 4273-4279.
NM Moore, NJ Lin, ND Gallant, ML Becker *Acta Biomaterialia*, 2011, *7*, 2091-2100.
NM Moore, NJ Lin, ND Gallant, ML Becker *Biomaterials*, 2010*, 31,* 1604-1611.

Bone Morphogenic Protein-2 (BMP-2)

N_3 –Linker- KIPKASSVPTELSAISTLYL

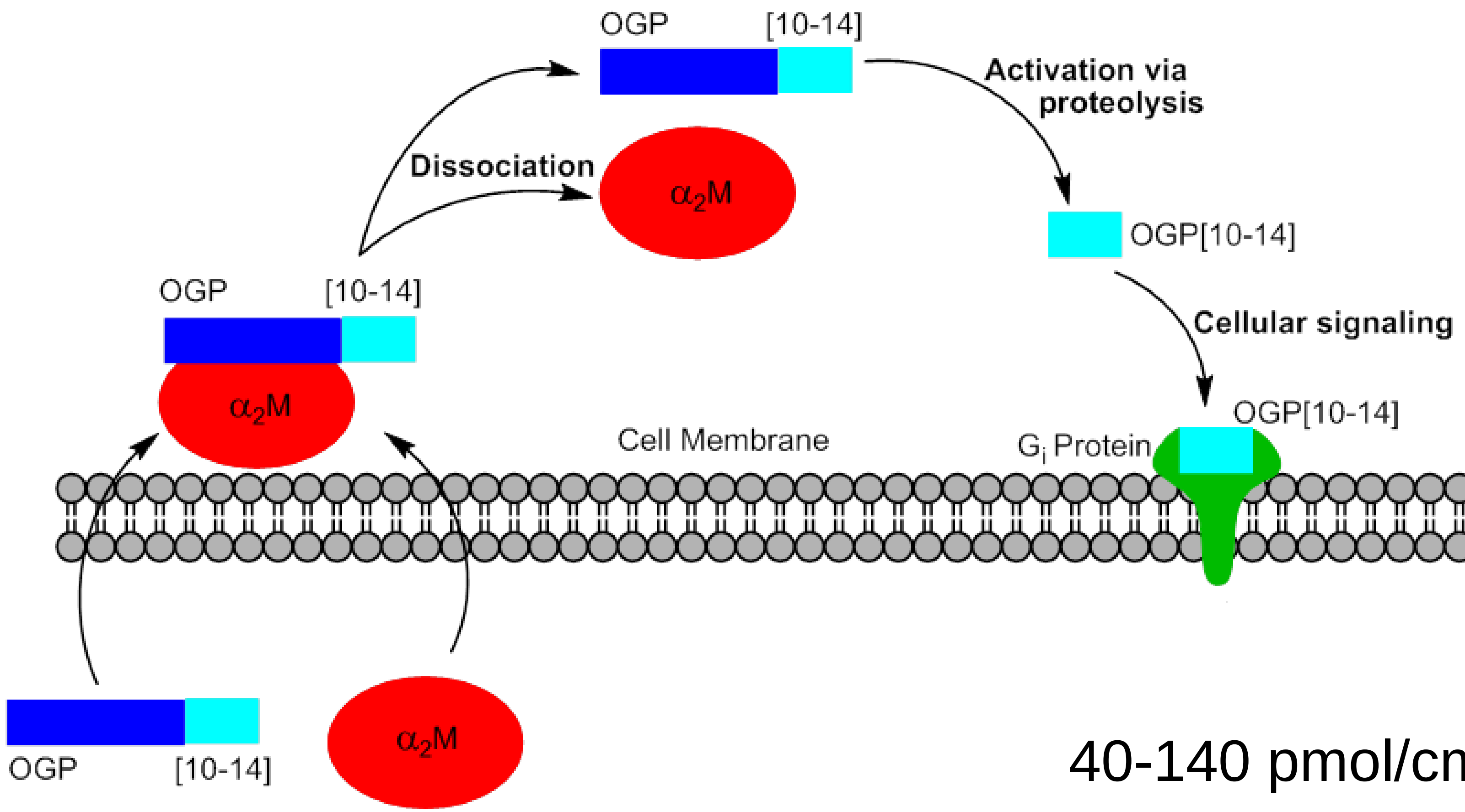


BMP binding to the heterodimeric receptor induce the phosphorylation and translocation of SMADS into the nucleus

NM Moore, NJ Lin, ND Gallant, ML Becker, *Biomaterials*. **2010,** *31,* 1604-1611
ND Gallant, KA Lavery, EJ Amis, ML Becker, *Advanced Materials*, **2007**, *19(7)*, 965-969.

Osteogenic Growth Peptide (OGP)

H-Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly-OH

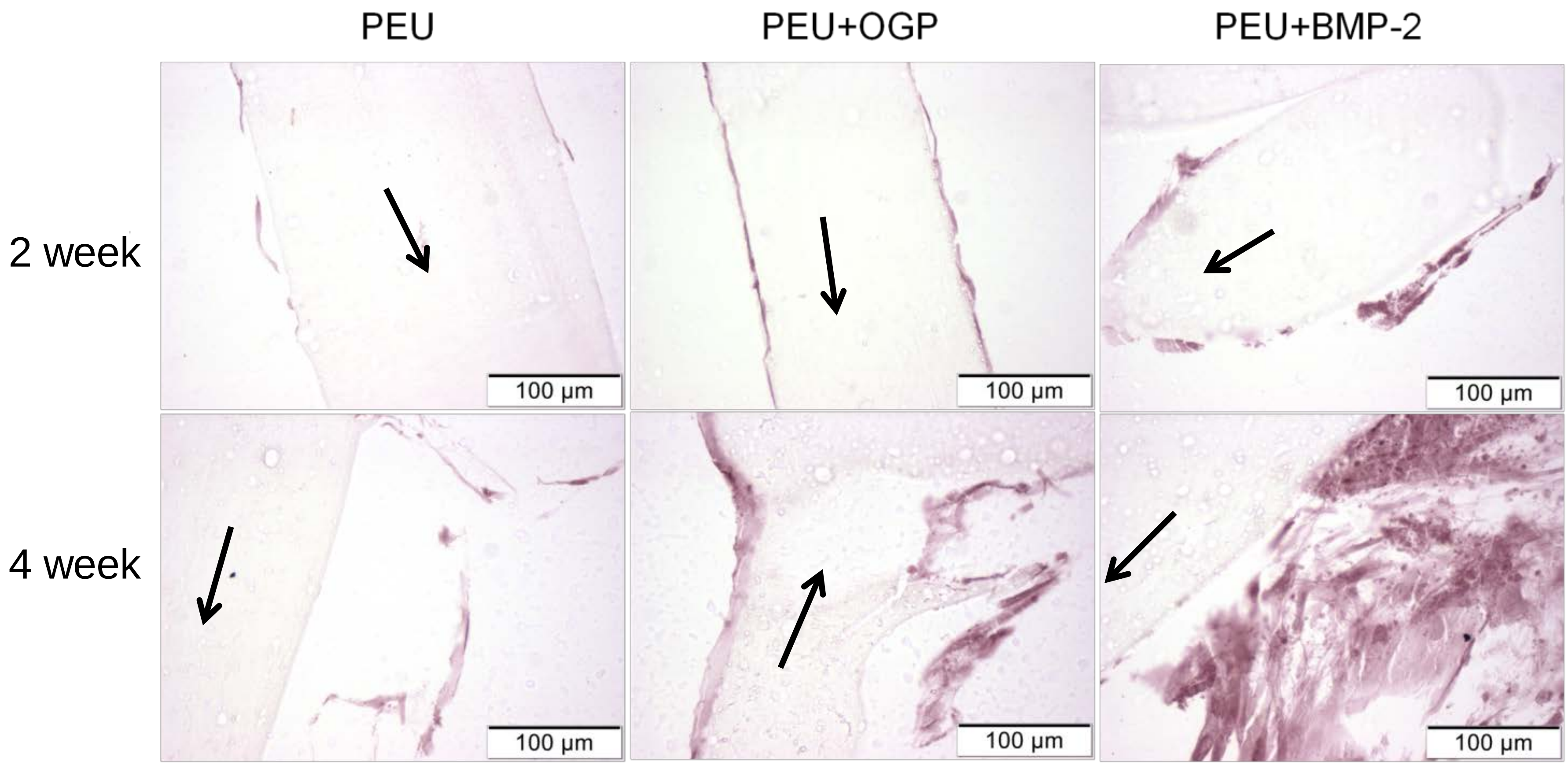


40-140 pmol/cm^2

Active domain drives mitogenic activity and differentiation of osteoblast cell lines

GM Policastro, ML Becker. *Wires Nanomed and Nanobiotechnology*. 2016, 8, 449-464.
NM Moore, NJ Lin, ND Gallant, ML Becker. *Biomaterials*. 2010, *31*, 1604-1611.

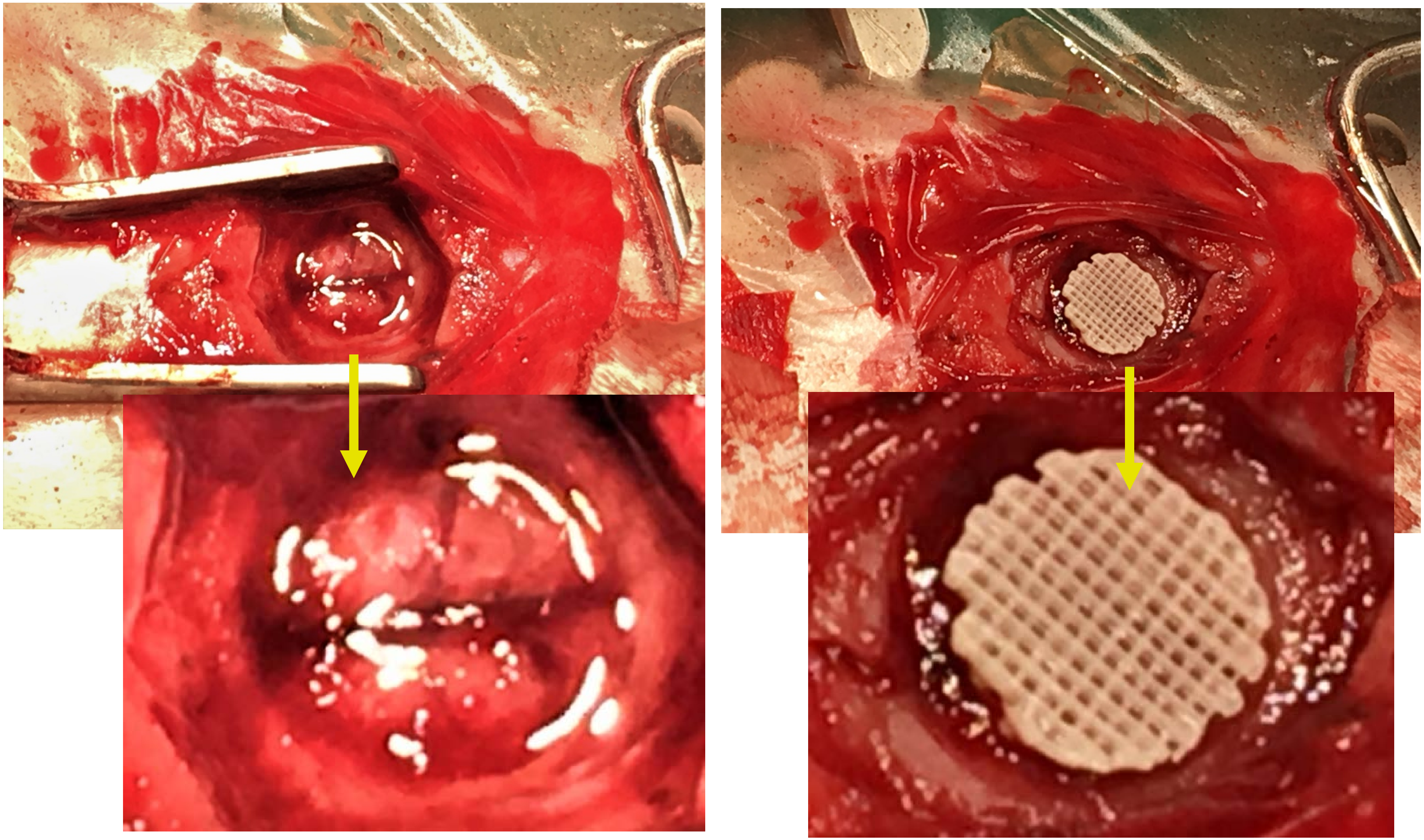
Mineralization of *h*MSCs on Scaffolds



Peptide introduction enhances *h*MSCs differentiation after 2 and 4 weeks culture.

S Li, Y Xu, J Yu, ML Becker* "Enhanced Osteogenic Activity of Poly(ester urea) Scaffolds using Facile Post-3D Printing Peptide Functionalization Strategies", *Biomaterials* 2017, *141,* 176-187.

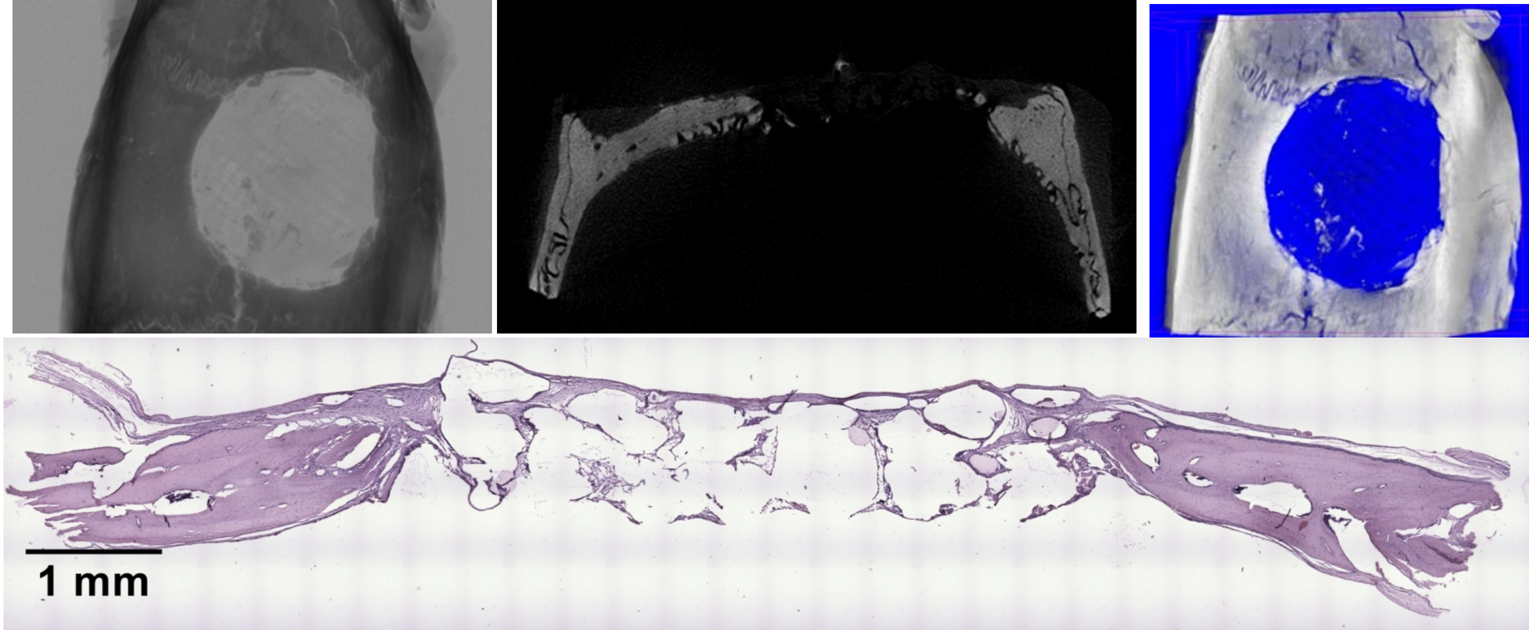
Critical Sized Rat Cranial Defect Repair



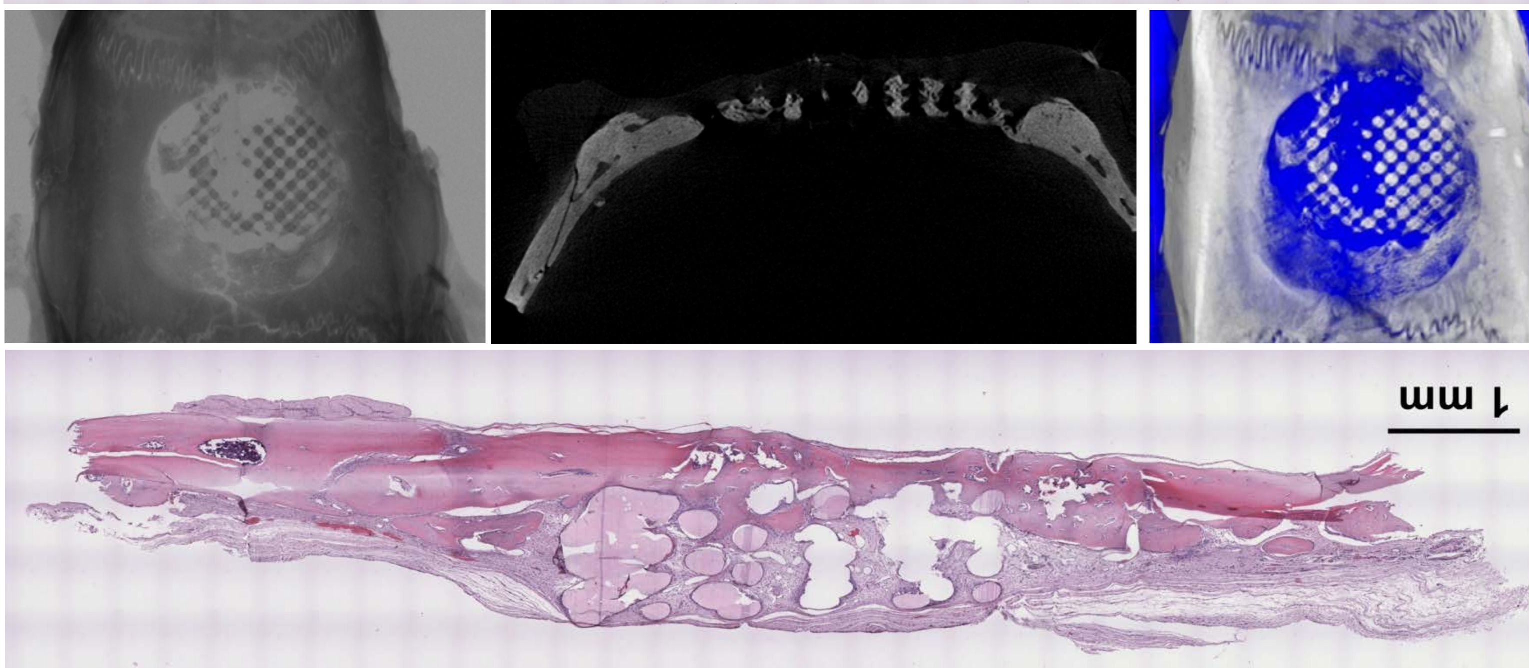
S Li, K Hagan, D Luong, Y Xu, J Yu, ML Becker, **2017**, *submitted*.

1 month

PEU

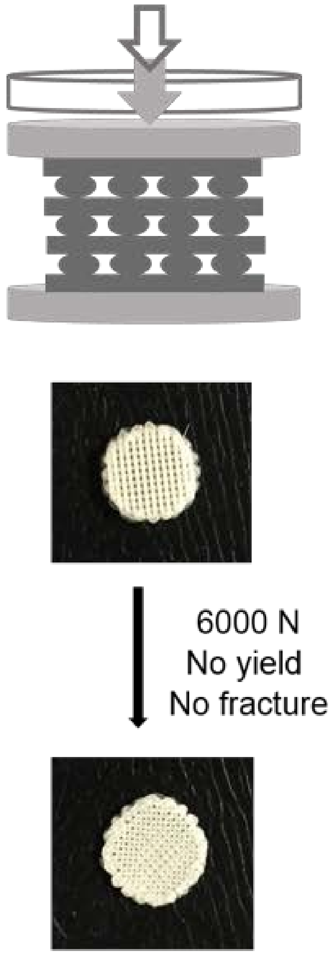


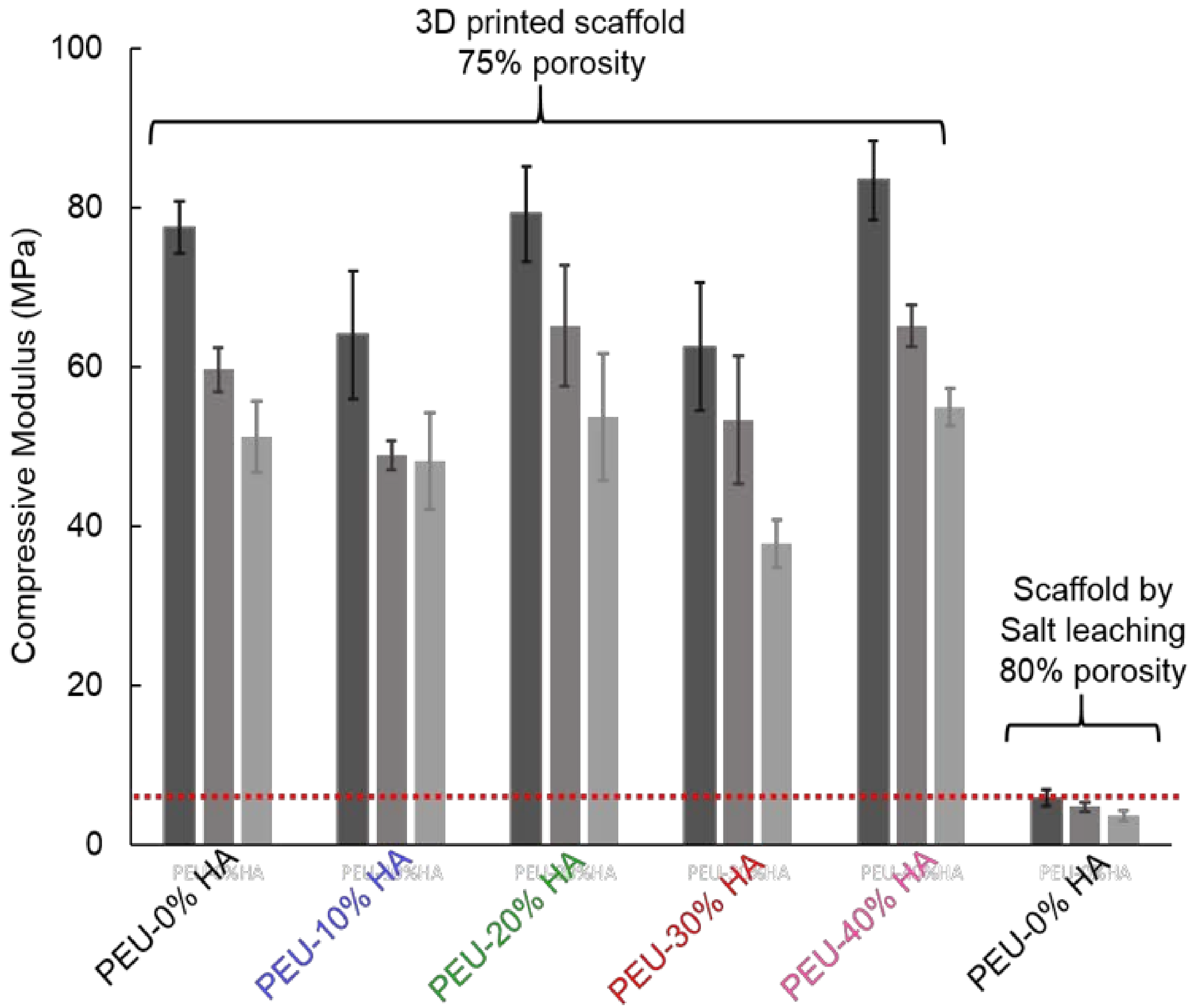
PEU w/ BMP-2 peptide



S Li, K Hagan, D Luong, Y Xu, J Yu, ML Becker, **2017**, *submitted*.

Are the scaffolds strong enough?





J Yu, Y Xu, S Li, GV Siefert, ML Becker* *Biomacromolecules* **2017**, *18 (12),* 4171–4183.

Conclusions:

Poly(ester urea)s have shown promise in a number of applications

Buffered Degradation is emerging success hypothesis

Functional Healing of an unsupported segmental defect is unprecedented

Highly tunable - mechanically & chemically & Functionally

Reactive handles survive filament generation and 3D printing.

The Patients are Waiting

Seeking Postdoctoral Scholars – Synthesis & Bioengineering (Bone & Drug Delivery)

Tomorrow's problems will not be solved by yesterday's materials

Becker Laboratory for Functional Biomaterials

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Laura Savriau
Karen Domino

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US Department of Defense – US Army MRMC W81XWH-15-1-0718
Air Force– Trust Initiative

Ohio Third Frontier – Innovation Platform, Ohio Opioid Challenge

Merck, Cook Biotech, Cook Medical, Viscus Biologics,
21st Century Medical Technologies
Knight Foundation, Kepley Foundation