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PRINCIPAL INVESTIGATOR: Warren Grayson, PhD

RECIPIENT: Johns Hopkins University

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

1. The number and severity of battlefield injuries to the craniofacial region increased significantly with the operations in Iraq and Afghanistan as has the survival of personnel with hemifacial injuries after ballistic trauma. Non-battlefield craniofacial injuries, including blunt trauma from motor vehicle accidents and falls, also provide a significant challenge in military operations and are relevant to the general population. Because of the complex nature of these injuries and the location in the craniofacial region, multiple tissue types suffer damage. These defects often involve a loss of maxillary and periorbital architecture resulting in poor malar projection, orbital dystopia, and visual sequelae. These craniofacial injuries often require numerous sequential complex surgeries that often do not achieve adequate aesthetic restoration or functional recovery. Therefore, there is a critical need for new solutions and improved surgical methods to treat these injuries.

2. This work will deliver GMP grade scaffolds comprised of human bone extracellular matrix (**ECM**) blended with polycaprolactone (**PCL**) into **hybrid ECM-PCL scaffolds** that can be 3D-printed into precise anatomical structures and effectively integrated with the patients' stromal vascular fraction cells for the treatment of geometrically complex bone defects in craniofacial trauma, focusing on the periorbital regions. This single distinct technology developed during Grant W81XWH-11-2-0022 will be integrated for this complex reconstruction of hard (bone) tissues in periorbital defects.

15. SUBJECT TERMS
Orbital reconstruction, bone graft, 3D-printing

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Background. The number and severity of battlefield injuries to the craniofacial region increased significantly with the operations in Iraq and Afghanistan as has the survival of personnel with hemifacial injuries after ballistic trauma. Non-battlefield craniofacial injuries, including blunt trauma from motor vehicle accidents and falls, also provide a significant challenge in military operations and are relevant to the general population. Because of the complex nature of these injuries and the location in the craniofacial region, multiple tissue types suffer damage. These defects often involve a loss of maxillary and periorbital architecture resulting in poor malar projection, orbital dystopia, and visual sequelae. These craniofacial injuries often require numerous sequential complex surgeries that often do not achieve adequate aesthetic restoration or functional recovery. Therefore, there is a critical need for new solutions and improved surgical methods to treat these injuries.

Objective/Hypothesis. This work will deliver GMP grade scaffolds comprised of human bone extracellular matrix (**ECM**) blended with polycaprolactone (**PCL**) into **hybrid ECM-PCL scaffolds** that can be 3D-printed into precise anatomical structures and effectively integrated with the patients' stromal vascular fraction cells for the treatment of geometrically complex bone defects in craniofacial trauma, focusing on the periorbital regions. This single distinct technology developed during Grant W81XWH-11-2-0022 will be integrated for this complex reconstruction of hard (bone) tissues in periorbital defects.

Specific Aims. Based on the critical military need for surgical management of complex craniofacial injuries including the mid-face orbital region, and the emphasis on translational approaches the following specific aims will be addressed:
Specific Aim 1. Develop ECM-PCL composite biodegradable scaffolds for reconstructing periorbital, bony defects. We will develop protocols for printing scaffolds with the appropriate mechanical and degradation properties.
Specific Aim 2. Integrate 3D-printed ECM-PCL scaffolds for regenerating vascularized bone in cranial (mouse) and periorbital (pig) bone defects. The ECM-PCL scaffolds will be integrated with human SVF for mouse studies. For pig studies, we will 3D-print anatomically-shaped ECM-PCL scaffolds for the treatment of zygomatic bone segmental defects in Yorkshire pigs with and without porcine SVF.
Specific Aim 3. Transition steps for GMP production of clinical-grade ECM-PCL scaffolds will be performed.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Bone defects, periorbital, stem cells, reconstruction, trauma, porcine, craniofacial, scaffold

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project? *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

<table>
<tr><th>Specific Aim 1</th><th>Timeline</th><th>Progress</th></tr>
<tr><td>Major Task 1: Develop defined bioactive, biodegradable ECM-PCL composite bone implants.</td><td>Months</td><td></td></tr>
<tr><td>Task 1A: Define the formulation (wt/wt ratios) of ECM-PCL scaffolds.</td><td></td><td></td></tr>
<tr><td>a. Evaluate quality of 3D prints as a function of ECM concentrations.</td><td>1-12</td><td>100%</td></tr>
<tr><td>b. Assess mechanics and degradation rates of ECM-PCL scaffolds with respect to ISO compliance.</td><td>1-12</td><td>100%</td></tr>
<tr><td>Task 1B: Assess biological characteristics of 3D-printed ECM-PCL scaffolds.</td><td></td><td></td></tr>
<tr><td>a. Perform proteomic analysis of bovine ECM pre- and post-printing.</td><td>1-24</td><td>100%</td></tr>
<tr><td>b. Cultivate human SVF in ECM-PCL scaffolds in vitro to assess osteoinductivity.</td><td>1-24</td><td>100%</td></tr>
<tr><td colspan="3">Milestone(s) Achieved: Task 1 will result in the optimized formulation of the ECM-PCL scaffolds based on bovine bone ECM to be 3D printed for use in the large animal periorbital defect.</td></tr>
<tr><td>Specific Aim 2</td><td></td><td></td></tr>
<tr><td>Major Task 2: Evaluation of scaffold technology for orbital bone reconstruction.</td><td></td><td></td></tr>
<tr><td>Task 2A: Calvarial bone defect reconstruction in murine model.</td><td></td><td></td></tr>
<tr><td>a. Combine bioscaffolds with human SVF to regenerate vascularized bone in 4-mm calvarial defect in immunocompromised mice.</td><td>13-24</td><td>100%</td></tr>
<tr><td>Task 2B: Periorbital bone defect reconstruction in porcine model.</td><td></td><td></td></tr>
<tr><td>a. Short-term (6 weeks) and long-term (12 months) term assessments of zygomatic reconstruction in pig model.
<table>
<tr><th>Groups</th><th>Defects per animal</th><th>No. of animals</th><th>Time-points</th><th>Evaluation</th></tr>
<tr><td>Empty Defect</td><td>2</td><td>2</td><td>12 months</td><td rowspan="2">•Computed Tomography (Pre-op, post-op, 6 weeks, 12 months)
•CBC/Chem Pre-op, 6 weeks, 12 months
•Tetracycline Administration Surgery
•Calcein Administration 6 weeks</td></tr>
<tr><td>Scaffold + Fibrin/SVF + PDGF</td><td>2</td><td>2</td><td>6 weeks</td></tr>
</table>
<table>
<tr><th>Groups</th><th>Defects per animal</th><th>No. of animals</th><th>Time-points</th><th>Evaluation</th></tr>
<tr><td>Scaffold + Fibrin/SVF</td><td>2</td><td>3</td><td rowspan="2">12 months</td><td rowspan="2">•Computed Tomography (Pre-op, post-op, 6 weeks, 12 months)
•CBC/Chem Pre-op, 6 weeks, 12 months
•Tetracycline Administration Surgery
•Calcein Administration 6 weeks</td></tr>
<tr><td>Scaffold + Fibrin/SVF + GF</td><td>2</td><td>3</td></tr>
</table>
</td><td>10-36</td><td>80%</td></tr>
<tr><td colspan="3">Milestone(s) Achieved: Task 2 will demonstrate the utility of the scaffolds with and without autologous cells and with/without growth factor to regenerate vascularized bone in the periorbital regions. We will obtain valuable insight on materials design leading to more standardized, reproducible implants that can be manufactured for large, anatomical reconstructions with no problems in donor tissue acquisition.</td></tr>
<tr><td>Specific Aim 3</td><td></td><td></td></tr>
<tr><td>Major Task 3: Translation of technology for orbital bone reconstruction.</td><td></td><td></td></tr>
<tr><td>Task 3A: Transition to clinical grade manufacturing.</td><td></td><td></td></tr>
<tr><td>a. Define SOPs for manufacturing scaffold.</td><td>13-24</td><td>80%</td></tr>
<tr><td>b. GMP production of anatomically shaped, ECM-PCL composite scaffolds.</td><td>13-36</td><td>10%</td></tr>
<tr><td colspan="3">Milestone(s) Achieved: Task 3 will establish the ground work for translation to a pre-clinical model and manufacture following GMP. Efficacy for composite tissue repair will be established along with translation.</td></tr>
</table>

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Here are the major accomplishments that were achieved during the period 30-Sep-2018 to 30-Sep-2019 in chronological order:

1. Design, fabrication and quality control of 3D printed custom implants
2. Successful pig surgeries validating the conceived full segmental bilateral zygomatic defect model
3. Monitoring bone growth in the operated pigs.

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1. Design, fabrication and quality control of 3D printed custom implants

One of the prime objectives of this ongoing study is to develop a protocol to fabricate custom implants which can be used as a substitute for autografts for treating critical sized bone defects. The challenge arises from variability in patient's anatomy as well as size and location of the defect. Herein we have designed and demonstrated a strategy for custom implant fabrication which opens up the possibility of a point of care treatment.

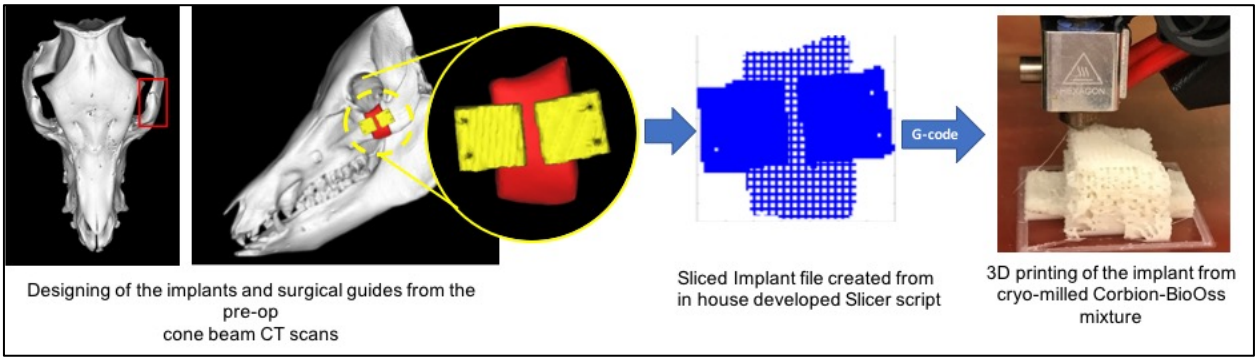

Figure 1. Design and 3D Printing of Implants: Workflow depicting the process of designing a custom implant from pre-op CT scans using MIMICS and 3D printing the designed implant.

Method:

Pre-op CT scans of the 10 pigs were performed approximately a month before the day of surgery. The pre-op CTs were used as a template for designing custom surgical guides and implants for each of the six pigs (2 implants/pig) using MIMICS software. Once the implants were designed, they were exported out of MIMICS in the form of slices (.bmp files) which could then be imported by a MATLAB script and generate a 3D volume in space of the implant i.e. a 3D matrix. Each elementary unit of this 3D matrix i.e. a voxel contains the greyscale values from the CT scan (spatially distributed within the 3D array) which is proportional to local mineral density of the bone. This 3D matrix is then discretized with each of its elements (or voxels) assigned/tagged a value for e.g. 0, 0.7 or 1 where 0, 0.7 and 1 imply empty space, body of the implant and fixation tab respectively. This discretized matrix is then passed into an inhouse developed Slicer script which slices the implant volume incorporating desired porosity at right locations

and creates the print path instructions for the 3D printer and these instructions are stored in the form of G-code (.gcode file). The g-code can be read using a 3D printer slicing software like CURA (opensource) and relay the print path instruction to the 3D printer. The nozzle of the 3D printer draws in either ABS or custom Corbion-BioOss (PCL-DCB) filaments and prints the implant. The above-mentioned workflow can be better visualized in Figure 1.

As a quick check to assess whether the implant design can be actually printed by the 3D printer, we first print a dummy implant with ABS plastic as it is a much cheaper option and print times are relatively lower. This step allows us to check whether the 3D printer can perform all the instructions on the print path effectively i.e. whether the implant design entails any tight bends, arcs, features smaller than the printer resolution and can't be captured by the movement of the print nozzle. If the ABS print fails to capture all the details of the implant design, then we need to go back to the pre-op CT scans and redesign or modify the implant. However, if the ABS print is successful, we go ahead and print with the Corbion-BioOss filaments.

Post printing the implants undergo a series of assessment both physical and computational in order to ensure that the implant dimensions and properties closely match with that of the desired custom implant. Basically, after 3D printing the implants, they undergo a thorough visual inspection to check for artifacts at the extremities, prominence or burn on the surface of the implant due to contact/dragging with the hot nozzle just above the print layer. Thereafter, a CT scan (cone beam CT) of the printed implants is performed and the reconstructed 3D image of it is analyzed digitally (with MIMICS software) to determine properties like porosity, pore connectivity, pore volume, etc. and the data is recorded. Physical print quality assessment data of the implants printed from ABS like dimensions of the extremities (anterior/posterior, span, fixation tab width) are compared with that of the implant design and are tabulated. This serve towards record-keeping as well as a checkpoint before proceeding towards printing the actual Corbion-BioOss (PCL-DCB) implants.

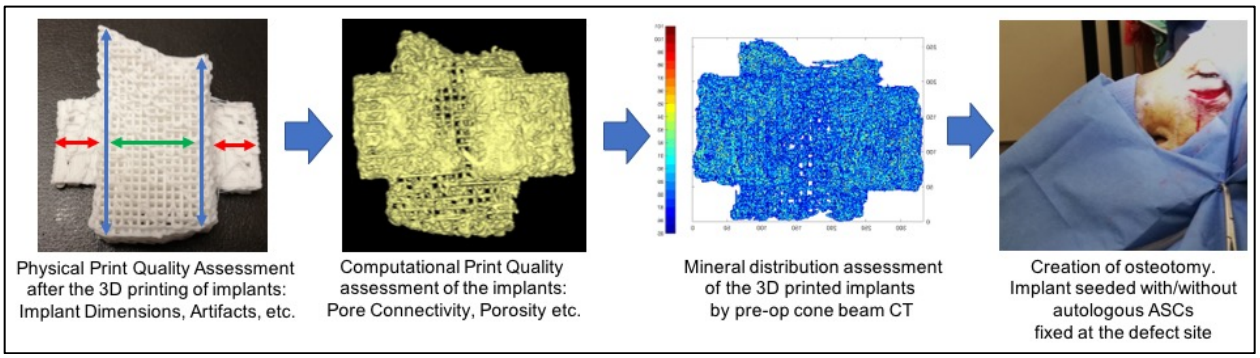

Figure 2. Print Quality Assessment of the 3D printed implants: Post printing the custom implants undergo a series of physical and computational examination to ensure that the design specification closely matches that with the dimensions of the printed scaffolds and also uniform mineral distribution throughout the scaffold.

As a second level of check, the 3D reconstruction of the implants from the CT scan is imported by a MATLAB script which slices/divides the reconstructed implant volume into several sub-regions and estimates the average mineral density (arbitrary units). This helps to ensure that the BioOss (or the decellularized bone particles) is uniformly distributed throughout the implant body. Any localized concentration of BioOss within the implant body can be easily detected as hotspots in the heatmap of the sub-regions generated by the MATLAB script. Moreover, the script can also calculate features like the implant volume, the volume of total pores within the implant, dimensions of the extremities of the implant (span, height/width, etc.) which can be compared with the corresponding values obtained from MIMICS software. This helps to validate the print and quality control assessment protocol. The above-mentioned steps are depicted in figure 2. In addition, pore volume calculated from the script helps us to get a better idea of how many cells can be seeded into the scaffolds, given a fixed volume of cell laden hydrogel (Tisseel) with a fixed cell density.

Pig ID	Implant Side	Volume (mm^3)	Min (GV)	Max (GV)	Avg (GV)	STD (GV)	Pore Interconnectivity	Average Pore Size (mm)	Surface Area (mm^3)	Specific Surface Area Calculated (mm)	Effective Porosity	Design Effective Porosity	Material Volume (mm3)	Design Material Volume (mm3)	Pore Volume (mm3)	Design Pore Volume (mm3)
3407	Left	2546	829	2270	1405	253.9	100%	0.7819	7144	2.81	40%	44%	2,538	2,557	1,665	1,977
3407	Right	2175	820	2760	1329	258.7	100%	0.7562	7238	3.33	44%	42%	2,170	2,459	1,700	1,778
3407	Right-Extra	2589	827	2243	1420	265.2	100%	0.7959	7413	2.86						
3412	Left	2150	813	2392	1390	257	100%	0.8106	6167	2.87	39%	44%	2,145	2,351	1,383	1,842
3412	Right	2231	828	2199	1398	253.3	100%	0.84	6287	2.82	40%	43%	2,224	2,428	1,486	1,813
3413	Left	2106	808	2375	1384	259.3	100%	0.8799	6127	2.91	40%	46%	2,102	2,184	1,389	1,823
3413	Right	2312	820	2190	1403	251.1	100%	0.82	6173	2.67	37%	42%	2,305	2,086	1,350	1,534
3422	Left	2946	802	3055	1395	264.2	100%	0.8048	8392	2.85	38%	46%	2,943	2,564	1,798	2,221
3422	Right	3136	825	2390	1383	254	100%	0.7717	9504	3.03	41%	48%	3,129	2,894	2,184	2,626
3441	Left	2532	804	2488	1433	261.2	100%	0.7908	5949	2.35	31%	43%	2,525	2,053	1,118	1,537
3441	Right	2529	804	2756	1445	262.6	100%	0.7588	5685	2.25	29%	40%	2,522	2,080	1,045	1,400
3447	Left	2452	836	2333	1421	254.2	100%	0.7919	6753	2.75	37%	45%	2,445	2,148	1,407	1,764
3447	Left-Extra	2376	838	2196	1417	256	100%	0.7952	6830	2.87						
3447	Right	2339	837	3318	1433	254.6	100%	0.8091	6235	2.67	36%	44%	2,332	2,090	1,301	1,639
		Measured Mask Properties (Printed Implant)					Pore Measurements (Printed Implant)				Theoretical Pore and Volume (Implant Design)					
		Estimated using MIMICS software									Estimated using MATLAB script					

Table 1: Comparison of implant design specifications (obtained using MATLAB Slicer script) with the dimensions/properties of the printed implants (determined from the cone beam CT scans of the 3D printed custom scaffolds).

Various design features of the all the 14 implants (2 extra) fabricated for the surgeries (of the 6 pigs that took place in June'19) like implant volume, pore volume, porosity, pore dimensions etc. have been summarized in Table 1. They were determined using the cone beam CT scans of 3D printed implants. The theoretical value of the above features were estimated using the inhouse developed MATALB Slicer script. Comparison of the theoretical values and the measured values of few key features have been depicted in figure 3.

It can be observed that all the implants had a very uniform pore dimension matching closely with that of the design (0.8mm) (figure 3a). The mineral distribution has been uniform throughout all the scaffold with an average score of ~1400 and the distribution is pretty narrow implying that there was no local concentration of decellularized bone matrix (figure 3b). Also, the design specification like porosity and material volume have been in good agreement with the features of the 3D printed implants, however the porosity showed a higher deviation (figure 3c).

Figure 3: Printed implant features for print quality assessment: a) Average pore size and b) Mineral distribution determined from cone beam CT scans of 3D printed implants. c) Deviation in the features of the 3D printed implants form that of the desired design specification.

2. Successful pig surgeries validating the conceived full segmental bilateral zygomatic defect model

Experimental Groups	Animals/ group (n)	CT Time Points	End of Study	Evaluation
Empty	2	Pre-Op, Post-Op, 6wk, 12 wk, 24wk, 48wk	48 weeks	Bone Formation (CT) Histology Mechanical strength Blood Work
Acellular	2	Pre-Op, Post-Op, 6wk, 12 wk, 24wk, 48wk	48 weeks	
Cellular	2	Pre-Op, Post-Op, 6wk	6 weeks	
	4	Pre-Op, Post-Op, 6wk, 12 wk, 24wk, 48wk	48 weeks	

Table 2: Summary of all the experimental groups namely highlighting the evaluations that will be carried out along with the CT time points. Yellow shading indicates the animals which underwent surgery in December'18 and the ones in green indicate that of June'19.

Having been able to design the custom implants, the next challenge would be to successfully secure the implants at the site of the defect ensuring that the implants closely match the natural anatomical contour and don't cause post-surgical morbidity or discomfort. To demonstrate the feasibility and robustness of the method, surgeries were performed on a set of 10 pigs at LSU in 2 rounds: the first set comprising of 4 pigs (Pig ID: 3077, 3097, 3105, 3134) in December'18 and the second set comprising the rest of 6 pigs (ID: 3407, 3422, 3412, 3413, 3441, 3447, 3412) in June'19. Briefly, a 2cm full thickness, segmental defect was created bilaterally in the zygomatic bone of each of the pigs. The pigs were divided into three groups namely empty, cellular and acellular. The acellular and cellular groups received the 3D printed custom implants with or without stromal vascular fraction (SVF) harvested from the corresponding animals respectively. The empty group was left untreated and received no implants. All the groups and the evaluations are summarized in Table 2.

In Dec' 18, the first round of surgeries was performed essentially as a pilot study to assess the feasibility of the method and to identify potential sources of error/complications which may include anything from hygiene to logistics. Ostectomy was performed on 2 pigs (ID: 3077,3097) and received no implants. These pigs would serve as the control (Empty) group for the study. 2 more pigs (ID: 3105, 3134), part of the cellular group underwent ostectomy and received implants with autologous SVF in (Tisseel) hydrogel.

The next round of surgeries was performed in June'19 which saw 6 pigs undergoing osteotomy. For the acellular group (Pig ID: 3407, 3441) the implants were placed in the defect and injected with 5ml of Tisseel (hydrogel) only whereas for the cellular groups (Pig ID: 3412, 3413, 3422, 3447) the implants were injected with 5ml of Tisseel (hydrogel) containing autologous SVF from the corresponding pigs. Post surgery, the pigs resumed normal activities and were regularly observed. Evaluations like CT scans, blood work were carried out at the allotted time points and evaluations like mechanical strength and histology would be performed once the implants are harvested when the end point is reached.

The detailed surgical procedure performed on each pig (in June'19) can be found below:

Pig ID: 3407
Treatment: Bilateral Zygomatic Ostectomies + Bilateral Implants, without Autologous Cells
Date: Wednesday 12 June 2019
Surgeons: M. Grant, J. Lopez, M. Lopez
Anesthetist: Mendoza
Total Surgery Time: 1 hr, 30 min

The face of the pig was prepped in standard fashion. Attention was turned to the to the right side. A 6 cm incision was drawn over the body of the zygoma with a marking pen. Using a #20 blade the incision was made through the skin and subcutaneous tissue. Hemostasis was achieved using monopolar cautery. The periosteum over the body of the zygoma was opened sharply 1 cm below the inferior orbital rim, and a sub-periosteal dissection performed, exposing the body of the zygoma, inferior orbital rim and anterior 1 cm of the orbital floor. A 2 cm right-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Hemostasis was achieved with monopolar cautery. The wound was irrigated copiously with saline, and a saline-soaked gaze was placed in the osteotomy.

Attention was turned to the left side where the identical dissection was performed. A 2 cm left-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour

matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Custom designed porous implants were then placed in the defects. They were fixated with four 2.0 x 8 mm screws. Following this 5 cc of Tisseel without fat-derived stem cells was injected into the implant. The periosteum was closed over the implant with interrupted 0 PDS bilaterally. The wounds were then closed in the subcutaneous layer with interrupted 0 PDS sutures, and the skin closed with a running intra-cuticular 2-0 Biosyn, followed by surgical cutaneous glue. The procedure was tolerated well, and the pig taken to the CT scanner for post-operative imaging, and then to the recovery area.

Pig ID: 3422
Treatment: Bilateral Zygomatic Ostectomies + Bilateral Implants + Autologous ASCs
Date: Wednesday 12 June 2019
Surgeons: M. Grant, J. Lopez, M. Lopez
Anesthetist: Mendoza
Total Surgery Time: 2 hr 10 min

The pig was generally anesthetized (See anesthesia record) and placed in ventral recumbency. The lumbar paravertebral area was aseptically prepared and draped. One 6 cm skin incision was made approximately 3 cm ventral to the dorsal midline over the area superficial to L3 – L6. The subcutaneous fat was sharply dissected and excised. Subcutaneous tissue was approximated with #0 PDS in a simple interrupted pattern. Skin was opposed with #2 nylon in vertical mattress sutures.

The face of the pig was prepped in standard fashion. Attention was turned to the to the right side. A 6 cm incision was drawn over the body of the zygoma with a marking pen. Using a #20 blade the incision was made through the skin and subcutaneous tissue. Hemostasis was achieved using monopolar cautery. The periosteum over the body of the zygoma was opened sharply 1 cm below the inferior orbital rim, and a sub-periosteal dissection performed, exposing the body of the zygoma, inferior orbital rim and anterior 1 cm of the orbital floor. A 2 cm right-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Hemostasis was achieved with monopolar cautery. The wound was irrigated copiously with saline, and a saline-soaked gaze was placed in the osteotomy. Attention was turned to the left side where the identical dissection was performed. A 2 cm left-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Custom designed porous implants were then placed in the defects. They were fixated with four 2.0 x 8 mm screws. Following this 5 cc of a 50/50 mix of fat-derived stem cells and Tisseel were injected into the implant. The periosteum was closed over the implant with interrupted 0 PDS bilaterally. The wounds were then closed in the subcutaneous layer with interrupted 0 PDS sutures, and the skin closed with a running intra-cuticular 2-0 Biosyn, followed by surgical cutaneous glue. The procedure was tolerated well, and the pig taken to the CT scanner for post-operative imaging, and then to the recovery area.

Pig ID: 3413
Treatment: Bilateral Zygomatic Ostectomies + Bilateral Implants + Autologous Cells
Date: Thursday 13 June 2019
Surgeons: M. Grant, J. Lopez, M. Lopez
Anesthetist: Mendoza
Total Surgery Time: 2 hr 30 min

The pig was generally anesthetized (See anesthesia record) and placed in ventral recumbency. The lumbar paravertebral area was aseptically prepared and draped. One 6 cm skin incision was made approximately 3 cm ventral to the dorsal midline over the area superficial to L3 – L6. The subcutaneous fat was sharply dissected and excised. Subcutaneous tissue was approximated with #0 PDS in a simple interrupted pattern. Skin was opposed with #2 nylon in vertical mattress sutures.

The face of the pig was prepped in standard fashion. Attention was turned to the to the right side. A 6 cm incision was drawn over the body of the zygoma with a marking pen. Using a #20 blade the incision was made through the skin and subcutaneous tissue. Hemostasis was achieved using monopolar cautery. The periosteum over the body of the zygoma was opened sharply 1 cm below the inferior orbital rim, and a sub-periosteal dissection performed, exposing the body of the zygoma, inferior orbital rim and anterior 1 cm of the orbital floor. A 2 cm right-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Hemostasis was achieved with monopolar cautery. The wound was irrigated copiously with saline, and a saline-soaked gaze was placed in the osteotomy.

Attention was turned to the left side where the identical dissection was performed. A 2 cm left-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Custom designed porous implants were then placed in the defects. They were fixated with four 2.0 x 8 mm screws. Following this 5 cc of a 50/50 mix of fat-derived stem cells and Tisseel were injected into the implant. The periosteum was closed over the implant with interrupted 0 PDS bilaterally. The wounds were then closed in the subcutaneous layer with interrupted 0 PDS sutures, and the skin closed with a running intra-cuticular 2-0 Biosyn, followed by surgical cutaneous glue. The procedure was tolerated well, and the pig taken to the CT scanner for post-operative imaging, and then to the recovery area.

Pig ID: 3441
Treatment: Bilateral Zygomatic Ostectomies + Bilateral Implants, without Autologous Cells
Date: Thursday 13 June 2019
Surgeons: M. Grant, J. Lopez, M. Lopez
Anesthetist: Mendoza
Total Surgery Time: 1 hr 0 min

The face of the pig was prepped in standard fashion. Attention was turned to the to the right side. A 6 cm incision was drawn over the body of the zygoma with a marking pen. Using a #20 blade the incision was made through the skin and subcutaneous tissue. Hemostasis was achieved using monopolar cautery. The periosteum over the body of the zygoma was opened sharply 1 cm below the inferior orbital rim, and a sub-periosteal dissection performed, exposing the body of the zygoma, inferior orbital rim and anterior 1 cm of the orbital floor. A 2 cm right-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Hemostasis was achieved with monopolar cautery. The wound was irrigated copiously with saline, and a saline-soaked gaze was placed in the osteotomy.

Attention was turned to the left side where the identical dissection was performed. A 2 cm left-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide

was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Custom designed porous implants were then placed in the defects. They were fixated with four 2.0 x 8 mm screws. Following this 5 cc of Tisseel without fat-derived stem cells was injected into the implant. The periosteum was closed over the implant with interrupted 0 PDS bilaterally. The wounds were then closed in the subcutaneous layer with interrupted 0 PDS sutures, and the skin closed with a running intra-cuticular 2-0 Biosyn, followed by surgical cutaneous glue. The procedure was tolerated well, and the pig taken to the CT scanner for post-operative imaging, and then to the recovery area.

Pig ID: 3447
Treatment: Bilateral Zygomatic Ostectomies + Bilateral Implants + Autologous ASCs
Date: Friday 14 June 2019
Surgeons: M. Grant, J. Lopez, M. Lopez
Anesthetist: Mendoza
Total Surgery Time: 1 hr 30 min

The pig was generally anesthetized (See anesthesia record) and placed in ventral recumbency. The lumbar paravertebral area was aseptically prepared and draped. One 6 cm skin incision was made approximately 3 cm ventral to the dorsal midline over the area superficial to L3 – L6. The subcutaneous fat was sharply dissected and excised. Subcutaneous tissue was approximated with #0 PDS in a simple interrupted pattern. Skin was opposed with #2 nylon in vertical mattress sutures.

The face of the pig was prepped in standard fashion. Attention was turned to the to the right side. A 6 cm incision was drawn over the body of the zygoma with a marking pen. Using a #20 blade the incision was made through the skin and subcutaneous tissue. Hemostasis was achieved using monopolar cautery. The periosteum over the body of the zygoma was opened sharply 1 cm below the inferior orbital rim, and a sub-periosteal dissection performed, exposing the body of the zygoma, inferior orbital rim and anterior 1 cm of the orbital floor. A 2 cm right-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Hemostasis was achieved with monopolar cautery. The wound was irrigated copiously with saline, and a saline-soaked gaze was placed in the osteotomy. Attention was turned to the left side where the identical dissection was performed. A 2 cm left-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Custom designed porous implants were then placed in the defects. They were fixated with four 2.0 x 8 mm screws. Following this 5 cc of a 50/50 mix of fat-derived stem cells and Tisseel were injected into the implant. The periosteum was closed over the implant with interrupted 0 PDS bilaterally. The wounds were then closed in the subcutaneous layer with interrupted 0 PDS sutures, and the skin closed with a running intra-cuticular 2-0 Biosyn, followed by surgical cutaneous glue. The procedure was tolerated well, and the pig taken to the CT scanner for post-operative imaging, and then to the recovery area.

Pig ID: 3412
Treatment: Bilateral Zygomatic Ostectomies + Bilateral Implants + Autologous ASCs
Date: Friday 14 June 2019
Surgeons: M. Grant, J. Lopez, M. Lopez
Anesthetist: Mendoza
Total Surgery Time: 1 hr 30 min

The pig was generally anesthetized (See anesthesia record) and placed in ventral recumbency. The lumbar paravertebral area was aseptically prepared and draped. One 6 cm skin incision was made approximately 3 cm ventral to the dorsal midline over the area superficial to L3 – L6. The subcutaneous

fat was sharply dissected and excised. Subcutaneous tissue was approximated with #0 PDS in a simple interrupted pattern. Skin was opposed with #2 nylon in vertical mattress sutures.

The face of the pig was prepped in standard fashion. Attention was turned to the to the right side. A 6 cm incision was drawn over the body of the zygoma with a marking pen. Using a #20 blade the incision was made through the skin and subcutaneous tissue. Hemostasis was achieved using monopolar cautery. The periosteum over the body of the zygoma was opened sharply 1 cm below the inferior orbital rim, and a sub-periosteal dissection performed, exposing the body of the zygoma, inferior orbital rim and anterior 1 cm of the orbital floor. A 2 cm right-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Hemostasis was achieved with monopolar cautery. The wound was irrigated copiously with saline, and a saline-soaked gaze was placed in the osteotomy. Attention was turned to the left side where the identical dissection was performed. A 2 cm left-sided cutting guide, specific for this pig was placed over the zygoma and positioned appropriately using contour matching. The edges of the cutting guide were outlined on the bone with monopolar cautery. The guide was removed. A reciprocating saw with a 27 mm blade was used to perform a full thickness 2 cm osteotomy of the central body of the zygoma. Custom designed porous implants were then placed in the defects. They were fixated with four 2.0 x 8 mm screws. Following this 5 cc of a 50/50 mix of fat-derived stem cells and Tisseel were injected into the implant. The periosteum was closed over the implant with interrupted 0 PDS bilaterally. The wounds were then closed in the subcutaneous layer with interrupted 0 PDS sutures, and the skin closed with a running intra-cuticular 2-0 Biosyn, followed by surgical cutaneous glue. The procedure was tolerated well, and the pig taken to the CT scanner for post-operative imaging, and then to the recovery area.

3. Monitoring bone growth in the operated pigs

Post surgery, CT scans were performed on the pigs at the allotted time points and analysis was done to check the amount of bone growth in the defect site. MIMICS software was used for the purpose of thresholding and segmentation of the implant (secured at defect site) from the surrounding tissue. Screenshots of the implant were taken to qualitatively compare and assess bone growth at different time points as depicted in Figure 4.

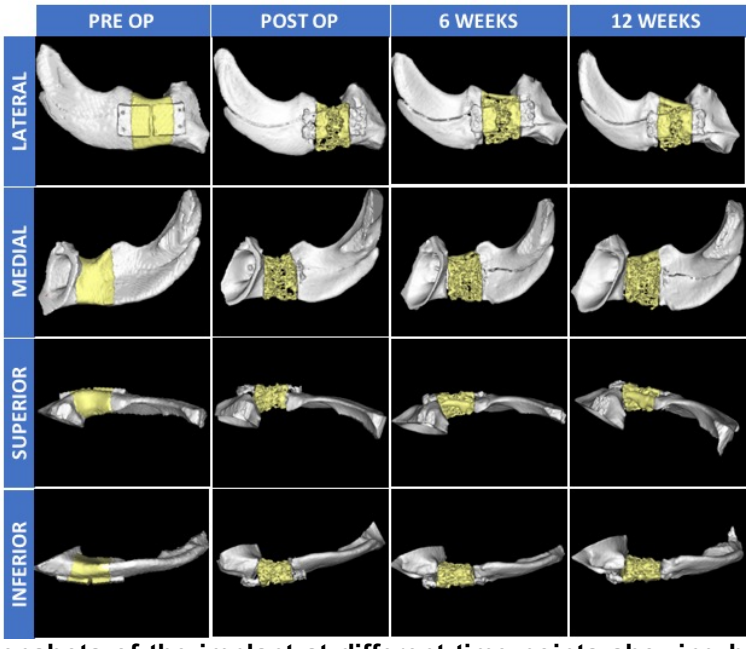

Figure 4: Screenshots of the implant at different time points showing bone growth at the defect site.

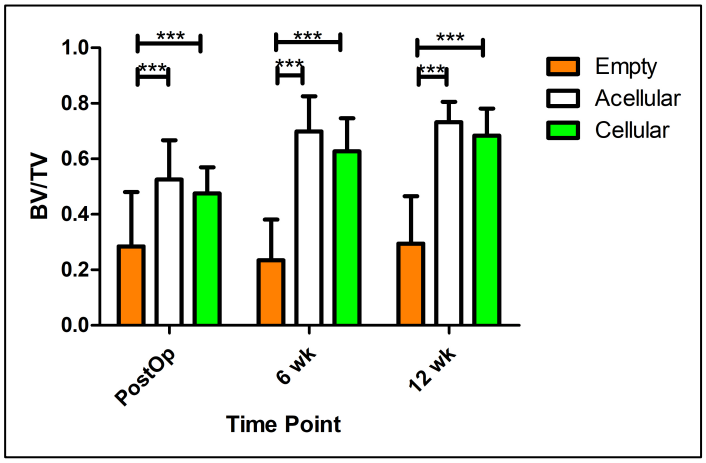

Figure 5: Comparison of bone regeneration across all experimental group at different time points.

In addition, Bone regeneration was quantified by means of cone beam CT scans. The ratio of (neo) Bone volume (BV) to total defect volume (TV) i.e. BV/TV has been used as a metric to compare bone regeneration among different groups. At 12 weeks, there has been no significant difference (two-way ANOVA test) between the cellular and acellular groups (Figure 5). Bone growth would be monitored till the end of study to get a better understanding of the effect of scaffold on bone regeneration at the site of defect.

Discussion of stated goals not met *These goals have been partially met due to delays in obtaining the IACUC and the subsequent ACURO approvals as well as due to delays in IRB approvals for the SVF isolation. However, we have since secured approvals for all of these elements and expect to achieve those goals over the next several months.*

There have been some other delays due to changes in plans, discussed in more detail in Section 5 below.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Courses: Srujan Singh took the course, Human Anatomy, as a part of Scientific Foundation of Medicine (SFM) offered at Johns Hopkins School of Medicine.

One-on-one work with mentor: The PI has one-on-one meetings each year with Srujan Singh to discuss project related elements as well as all other factors related to professional development.

Weekly or bi-weekly meetings: Srujan Singh meets weekly in a group meeting with the PI, Dr. Warren Grayson where progress on the project is discussed.

Meetings with clinicians: Srujan Singh has had meetings with Dr. Michael Grant to discuss on pig health post-surgery and to analyze the bone regeneration so far. Plans have been worked out regarding harvesting of the implants once the study reaches end point.

Conferences: Abstract titled "Point-of-Care Cell-Based Strategy for Treating Large Craniofacial Bone Defects" has been accepted for Oral presentation at the 11th World Biomaterials Congress 2020 and would be presented by Srujan Singh.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The findings so far were presented at 2019 Military Health System Research Symposium (MHSRS) with the poster title "Point-of-Care cell based strategy for treating large craniofacial bone defects"

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next quarter, we anticipate substantial progress on preliminary analysis of the data. We will be receiving CT scans for the 48wk time point for the pigs (Pig ID: 3077, 3097) operated in Dec'18 and 24 wk time point for the ones operated in June'19 (Pig ID: 3407, 3412, 3413, 3422, 3447). Comparison of bone regeneration would be done among the different groups. After reaching the end point (Pig ID: 3077, 3097), the implants would be harvested and they would undergo mechanical testing and histological analysis.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Being able to demonstrate the capability to fabricate custom implants, isolate autologous SVF (stromal vascular fraction) and surgically secure the SVF loaded implants in the defects is a very crucial step towards developing a point-of-care bone tissue engineering strategy to treat clinically relevant large sized cranio-facial defects.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Full patent application in review:
US Patent Application: 15/739946
Grayson WL, Hung BP, Elisseeff JH, 3D-Printed Extracellular Matrix Mixture and ECM Scaffolds Made with Polycaprolactone and decellularized bone.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  - Poster presentation at 2019 Military Health System Research Symposium (MHSRS) with the poster title "**Point-of-Care cell based strategy for treating large craniofacial bone defects**".
  - Article titled: **Comparison of Stromal Vascular Fraction and Passaged Adipose-Derived Stromal/Stem Cells as Point-of-Care Agents for Bone Regeneration** published in the journal **Tissue Engineering Part A**
  - Article titled: **scafSLICR: A MATLAB-based slicing algorithm to enable 3D-printing of tissue engineering scaffolds with heterogeneous porous microarchitecture** published in the journal **PLOS ONE**.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

1. Poster presentation at TERMIS-Americas: **E. L. Nyberg**, A. Farris, W. Grayson. *Assessing the Osteogenic Potential of Adipose-derived Stromal Vascular Fraction Cells.* Poster at the Tissue Engineering and Regenerative Medicine International Society Annual Conference. Charlotte, North Carolina. (2017) **** Winner, Student and Young Investigator Section ****

2. Poster presentation at JHU Department of Medicine Annual Retreat
   E. L. Nyberg, A. Farris, W. Grayson. *Assessing the Osteogenic Potential of Adipose-derived Stromal Vascular Fraction Cells*. (2018)
   **** Finalist, School of Engineering Trainee ****

3. Invited presentation at World Congress of Biomechanics.
   "Design and Manufacture of 3D-Printed Scaffolds for Regeneration of Massive Craniofacial Bone Loss", Ethan Nyberg, Aine O'Sullivan, Warren Grayson.

4. Oral presentation at the Northeast Bioengineering Conference "3D-Printing Heterogeneous Porous Patterns in Tissue Engineered Bone Scaffolds", Aine O'Sullivan, Ethan Nyberg, and Warren Grayson.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Full patent application in review: US Patent Application: 15/739946 Grayson WL, Hung BP, Elisseeff JH, 3D-Printed Extracellular Matrix Mixture and ECM Scaffolds Made with Polycaprolactone and decellularized bone.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*
Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award).*

Name	Project Role	*Researcher Identifier (e.g. ORCID ID):*	Nearest person month worked	Contribution to project
Warren Grayson	Principal Investigator	0000-0001-6099-6469	3	Oversaw animal studies
Mandi Lopez	Veterinarian		6	Managed swine surgery and housing
Michael Grant	Craniofacial Surgeon		2	Performed swine surgery (osteotomy)
Ashley Farris	Graduate Student		6	Cell Isolation
Srujan Singh	Graduate Student		12	Print Quality Assessment CT Scan Analysis
Aine O'Sullivan	Research Technologist		8	3D-printing systems and swine study Cell Isolation

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Active grants that have closed:
NSF CAREER Award - 1350554
Role: PI
Period: 05/15/14 – 05/14/19 0.6 CM $341,151
Program Officer: Steven Peretti, speretti@nsf.gov
Title: Modeling Stem Cell Decision Making During Vascularized Bone Development

Maryland Stem Cell Research Fund – Investigator-Initiated Research Proposal
Role: PI
Period: 07/01/16 – 06/30/19 1.2 CM $600,000
Program Officer: Dan Gincel, dgincel@tedco.md
Title: Engineering Contractile Muscle for Treatment of Volumetric Muscle Loss

New active grants:
Congressionally Directed Medical Research Programs
Role: Multi-PI (Contact PI)
Period: 9/30/17-9/29/20 1.2 CM $1,500,000
Scientific Officer: Nicole Enman nicole.m.enban.ctr@mail.mil
Title: Multi-Parametric Bioreactor for Functional Preservation of Vascularized Composite Allografts

Maryland Stem Cell Research Fund
Role: PI
Period: 07/01/18 – 02/29/20 0.6 CM $200,000
Program Officer: Dan Gincel, dgincel@tedco.md
Title: 3D-Printed, Oxygen-Delivering Scaffolds for Regenerating Vascularized Craniofacial Bone

R01 – National Institute of Dental and Craniofacial Research
Role: Contact PI (Grayson/Pathak)
Period: 04/01/19 – 03/31/24
Program Officer: Nadya Lumelsky nadyal@nidcr.nih.gov
Title: Oxygen-Eluting Scaffolds for Cranial Bone Regeneration

Maryland Stem Cell Research Fund – Discovery Award
Role: Co-I
Period: 07/01/19 – 06/30/21
Program Officer: Dan Gincel, dgincel@tedco.md
Title: An engineered orthogonal growth factor for targeted stimulation of bone repair

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Geistlich Pharma North America Inc.
202 Carnegie Center
Princeton, NJ 08540

Provided in-kind support by contributing Bio-Oss, the clinical grade bone ingredient in the implants.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

Nothing to Report.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Nothing to Report.