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TITLE: **Development of Smoothened Agonist Non-Phospholipid Liposomal Nanoparticles for Bone Repair**

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CONTRACTING ORGANIZATION: **Johns Hopkins University**
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14. ABSTRACT
Non-healing bone defects remain a significant problem for combat casualties and military veterans. A principle challenge is to develop therapeutic agents that safely and effectively improve growth and differentiation factor (GDF) based skeletal regeneration. The manipulation of Hedgehog signaling is a promising alternative to BMP2 for improved bone repair outcomes. Recently, we observed that the small molecule Hedgehog agonist SAG demonstrates pro-osteogenic / pro-vasculogenic effects to induce mouse calvarial defect healing. Independently, we have developed innately osteoinductive Stearylamine and Oxysterol (SA/Oxy) nanoparticles (NPs), and showed their high drug loading efficiency and synergistic osteoinductive potential with the small molecule SAG. In the current proposal, we will combine these recent breakthroughs to develop a next generation NP packaged small molecule as a bone graft substitute product to jumpstart endogenous bone repair.

15. SUBJECT TERMS
Bone repair, bone regeneration, bone tissue engineering, osteogenesis, Hedgehog signaling, Smoothened, Oxysterol

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1. INTRODUCTION

Non-healing bone injuries represent a source of morbidity for combat casualties and military veterans, exacting both a devastating individual toll on the lives affected as well as an enormous socioeconomic burden. The manipulation of Hedgehog (Hh) signaling is a promising alternative for improved bone regeneration. Our research group has shown that Hh signaling diverts mesenchymal stem cells (MSC) toward a bone-forming fate and away from competing cell fates. Moreover, the Hh activating small molecule SAG targets bone and vascular formation to induce bone healing. In a coordinate research effort, we have shown that non-phospholipid liposomes composed of Stearylamine and Oxysterol (SA/Oxy) have intrinsic bone inducing capabilities, and are well designed to deliver the small molecule SAG to sites of bone injury. In aggregate, the present proposal seeks to develop a nanoparticle (NP) delivered small molecule for faster, safer, and more efficacious bone repair than currently available treatment strategies. Here, we will perform key preclinical safety and efficacy studies for clinical translation of a nanoparticle packaged Hh small molecule for use as a widely applicable bone graft substitute, to be accomplished in two specific aims.

2. KEYWORDS

Non-phospholipid liposome, scaffold, smoothened agonist, hedgehog signaling, bone regeneration

3. ACCOMPLISHMENTS

What were the major goals of the project?

The project contains two aims as stated in the SOW:

Aim 1: Optimize SAG-loaded liposomal nanoparticles for mouse calvarial defect repair.
Aim 2: Determine the safety of SAG-loaded liposomal nanoparticles for mouse calvarial defect repair.

These aims are composed of two subtasks:

Subtask 1: Osteoinductive scaffold fabrication and batch validation
Subtask 2: SA/Oxy NP production and batch validation

What was accomplished under these goals?

- Develop non-phospholipid liposomal nanoparticles and validate bioactivity

Sterosome is a novel type of liposome formed from non-phospholipids. Sterosome with osteoinductive function was made with stearylamine (SA) and 20(s)-hydroxycholesterol (Oxy) using thin-film hydration method. Hydrodynamic characterization was studied by dynamic light scattering. Particle size, zeta potential, and poly dispersity index (PDI) are represented in **Table 1**. Hydrodynamic diameter of sterosome was measured as 157 ± 8 with a narrow distribution (PDI, 0.15 ± 0.03). Their ζ-potential was 66.9 ± 7.0.

Table 1. Hydrodynamic characterization of sterosomes

	Sterosome
Size (nm)	157 ± 8
PDI	0.15 ± 0.03
Zeta potential (mV)	66.9 ± 7.0

To examine the bioactivity of sterosomes, osteogenesis was examined in mouse bone marrow stromal cell line (BMSCs, D1 ORL, UVA [D1], D1 cell, CRL-12424). Osteogenic differentiation of MSCs in contact with sterosomes was evaluated by alkaline phosphatase (ALP) and mineralization staining and corresponding quantification from colorimetric assays. The ALP activity was intensified with increasing concentration of sterosomes in both ALP staining and colorimetric assay (**Figure 1**). Similar trends were confirmed for mineralization staining. Stronger red color appeared with increasing concentration of sterosomes (**Figure 2**). The quantification of ALP expression and mineralization showed results consistent with the corresponding staining.

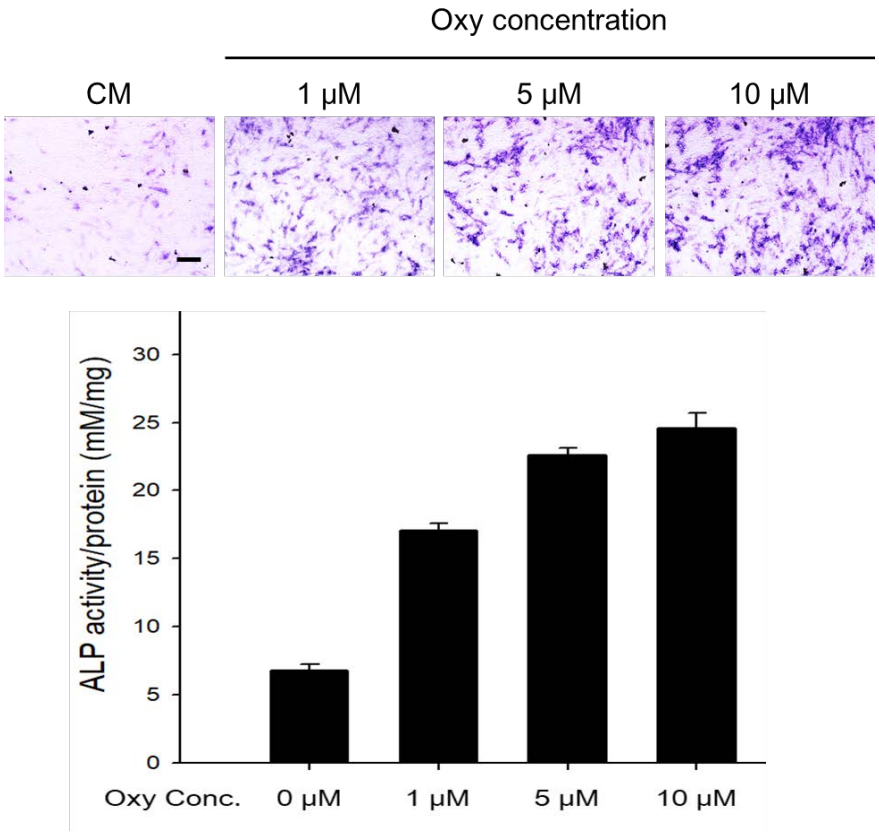

Figure 1. Bioactivity evaluation of sterosomes in BMSCs. ALP expression of BMSCs and colorimetric quantification of ALP activity for day 4.

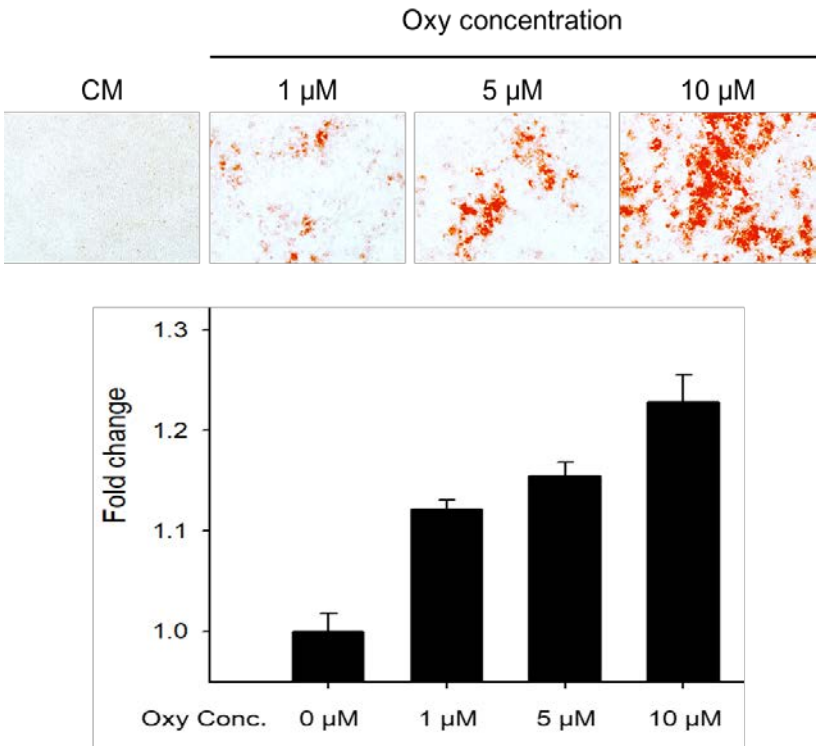

Figure 2. Bioactivity evaluation of sterosomes in BMSCs. Mineralization stained with Alizarin red S and colorimetric quantification of mineralization activity for day 14.

Key outcomes and conclusions:

Hydrodynamic characterizations ensure the validity of sterosome nanoparticles before progression to scaffold loading and *in vivo* application. Sterosome was consistently bioactive and its osteoinductive potential was verified by staining and colorimetric assay.

- Develop liposome immobilized 3D PLGA scaffolds

A liposome immobilized 3D PLGA scaffold was developed to achieve prolonged drug release via the polymerized dopamine intermediate with unprecedented simplicity. The application of polydopamine (PDA) has engaged enormous interest because of its bioinspired nature. Surface modification using PDA coatings has utilized intermediate as the immobilization of bioactive molecules and significantly promotes the adhesion and proliferation of cells with good biocompatibility and giving great potential for biomedical application.

Prior to immobilization with sterosome, 3D PLGA scaffolds were immersed with 5 g/L of dopamine solution under an alkaline oxidative condition (0.01 M Tris buffer, pH 8.5) for 1 hour to induce dopamine polymerization. Polymerized dopamine (pD) is known to bind tightly on solid surfaces through both covalent and noncovalent interactions, making a persistent layer that can serve as an intermediate for liposomal repository incorporation. After coating of 3D PLGA scaffold with pD, amine-functionalized sterosomes, which reacts with pD through Schiff-base formation and Michael-type addition under an oxidizing condition, were immobilized on pD surface of the scaffold.

After coating of PLGA NPs with pD, amine-functionalized sterosomes were immobilized with the 3D PLGA scaffolds via the pD surface under an oxidizing condition. Despite their great advantage as a drug carrier on surface of the scaffold, sterosomes do not readily serve intermediate surface to attach cells because of their hydrophobicity and electrical characteristic, such as high charge density, and thus require surface modification to attain the ability. Therefore, sterosome-coated 3D PLGA scaffold were modified with secondary pD coating, which can serve biocompatibility and aid controlled release of cargo in sterosomes.

The atomic composition of the major peaks (C 1s, O 1s, N 1s) were compared for a PLGA scaffold, polydopamine prime-coated PLGA scaffold, PLGA scaffold coated with sterosomes, PLGA scaffold coated with sterosomes and then polydopamine through X-ray photoelectron spectroscopy (XPS) analysis (**Figure 3 and Table 2**). All the functionalized scaffolds except for bare PLGA scaffold showed a peak at 397 eV, which corresponded to nitrogen (N 1s), verifying the presence of pD and sterosome layer. With each additional deposition step, the carbon signal was increasing, from 63.33% for the bare PLGA scaffold to 85.64% for the PLGA-pD-Lipo-pD scaffold, suggesting that the film thickness was increasing. In parallel, the O 1s signal and O/C ratio was decreasing with each deposition step. While no nitrogen was detected on the bare PLGA, pD and sterosome deposited scaffolds have shown N 1s peak because of the nitrogen in the sterosome as well as in pD with no remarkable N/C ratio change. The C 1s spectra of the scaffolds revealed substantial changes in the spectra after pD and sterosome deposition, which can be attributed to significant decrease in signals related to the PLGA at 285 eV (C-O), and 287 eV (O=C-O) (**Figure 4**). The combination of these results strongly suggests that the pD and sterosome is deposited successfully on surface of scaffolds via pD.

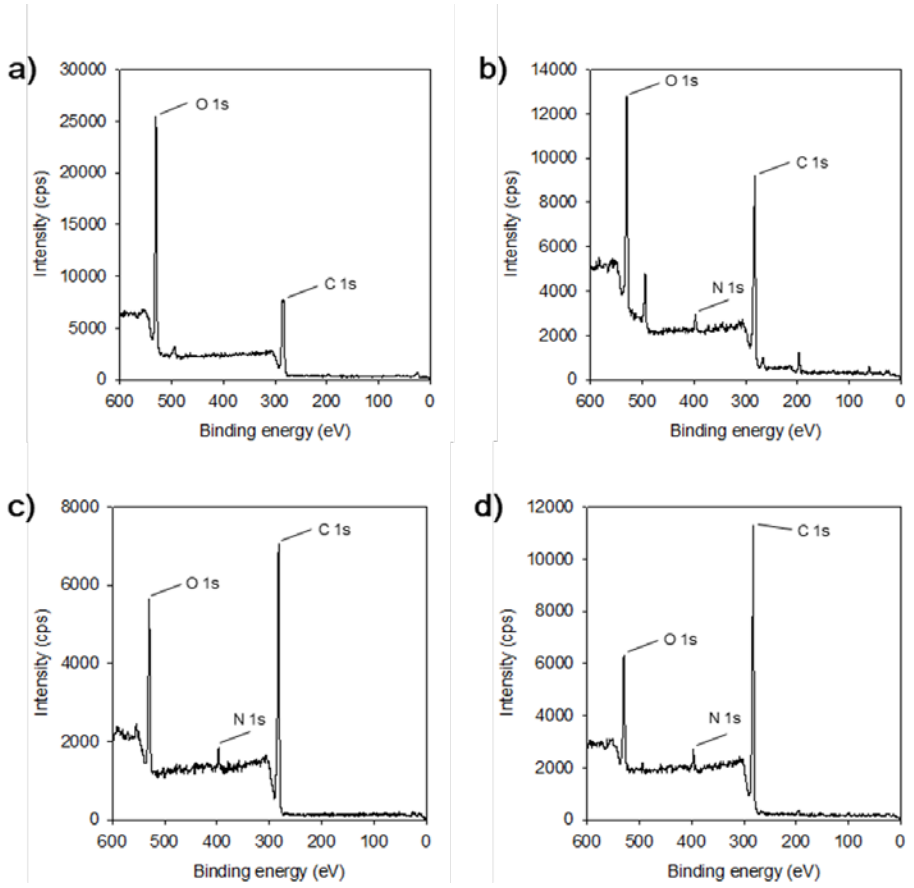

Figure 3. X-ray photoelectron spectroscopy (XPS) spectra of a) PLGA scaffold, b) polydopamine prime-coated PLGA scaffold, c) PLGA scaffold coated with sterosomes, d) PLGA scaffold coated with sterosomes and then polydopamine.

Table 1. Elemental composition and comparison of the atomic O/C and N/C ratios obtained by XPS analysis as received PLGA scaffold, polydopamine prime-coated PLGA scaffold, PLGA scaffold coated with sterosomes, PLGA scaffold coated with sterosomes and then polydopamine.

Substrate	Elemental composition* (%)			O/C	N/C
	C	O	N		
PLGA	63.33	36.67	-	0.58	0.00
PLGA-pD	73.22	24.49	2.28	0.33	0.03
PLGA-pD-Lipo	81.25	16.08	2.68	0.20	0.03
PLGA-pD-Lipo-pD	85.64	12.24	2.12	0.14	0.02

* XPS elemental composition was calculated from X-ray photoelectron spectroscopy survey scan spectra.

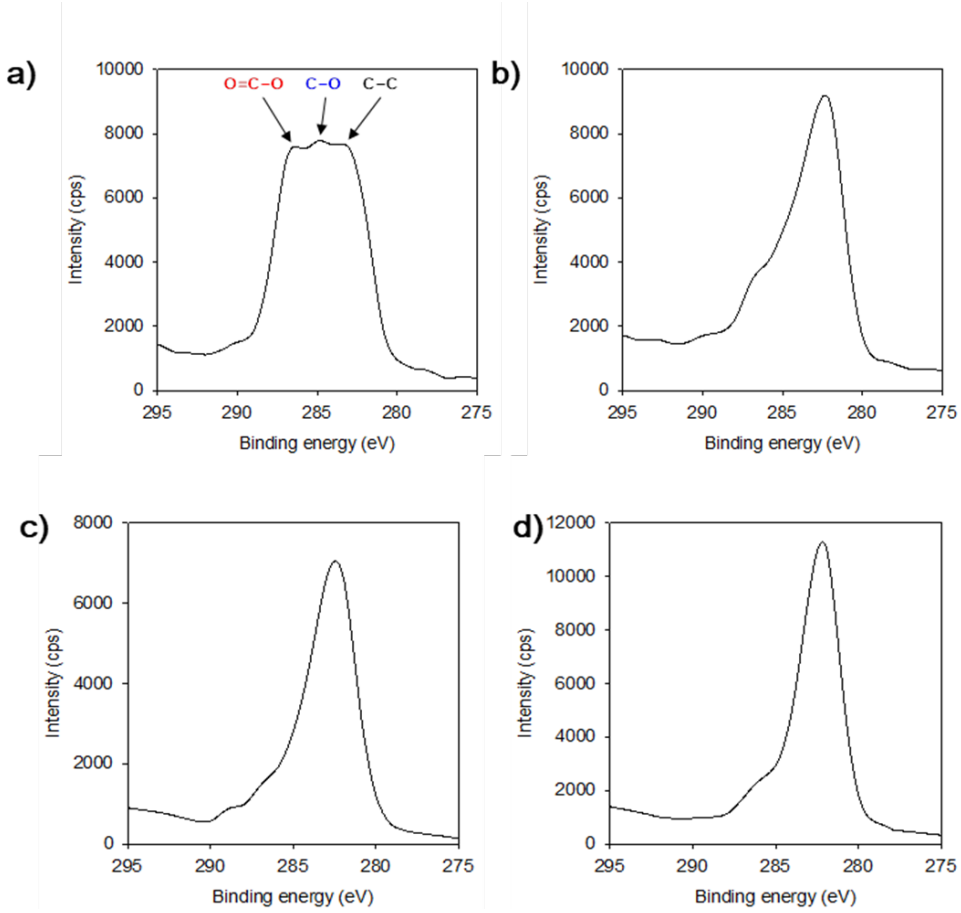

Figure 4. X-ray photoelectron spectroscopy (XPS) C 1s spectra of a) PLGA scaffold, b) polydopamine prime-coated PLGA scaffold, c) PLGA scaffold coated with sterosomes, d) PLGA scaffold coated with sterosomes and then polydopamine.

The scaffolds with immobilized pD and sterosomes were analyzed by scanning electron microscopy (SEM) to investigate surface morphology and roughness (**Figure 5**). The unmodified PLGA scaffolds exhibited smooth surface morphology, compared with the modified substrates. Surface

roughness increased by the sequential deposition of pD and sterosomes on PLGA scaffolds, which is related to increase of coating thickness.

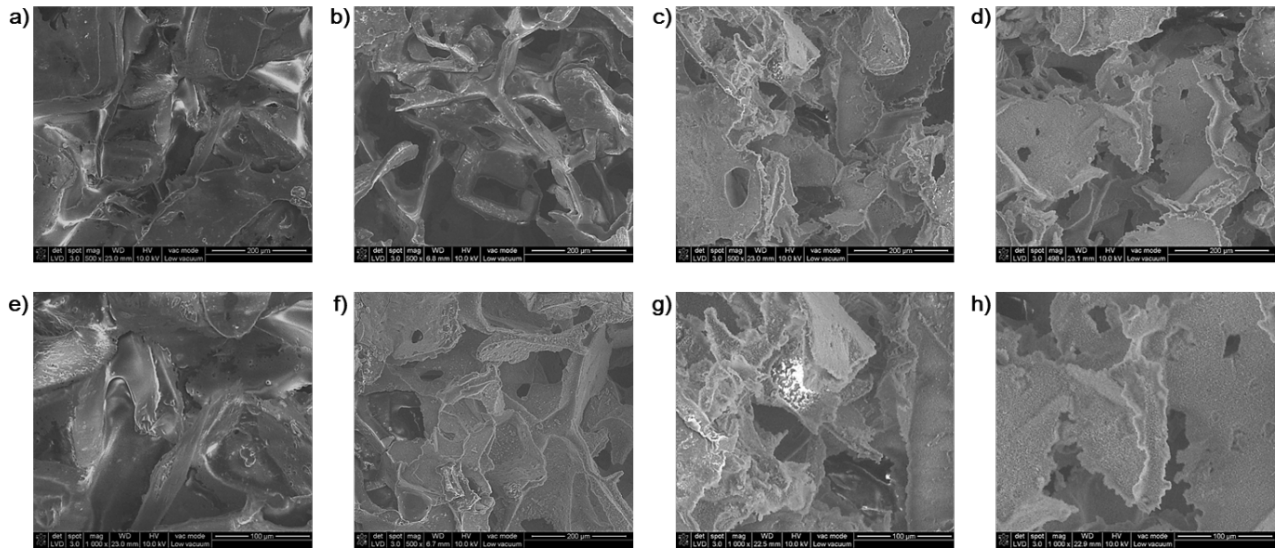

Figure 5. SEM image of a and e) PLGA scaffold, b and f) polydopamine prime-coated PLGA scaffold, c and g) PLGA scaffold coated with sterosomes, d and h) PLGA scaffold coated with sterosomes and then polydopamine.

To evaluate the efficiency of pD-mediated immobilization on surface of the scaffolds, Nile red (NR)-loaded sterosome (NR-sterosome) was used as a model liposome. NR is a fluorescent dye as a hydrophobic model drug (**Figure 6**). When bare and pD coated PLGA scaffolds was incubated with NR-sterosome (1 g/L) for 1 hour, pD coated PLGA scaffolds increased the amount of immobilized sterosomes by 3.5-fold compared with that of bare PLGA scaffolds. This indicated that pD-mediated immobilization was highly efficient and stable for modification of the scaffold surface.

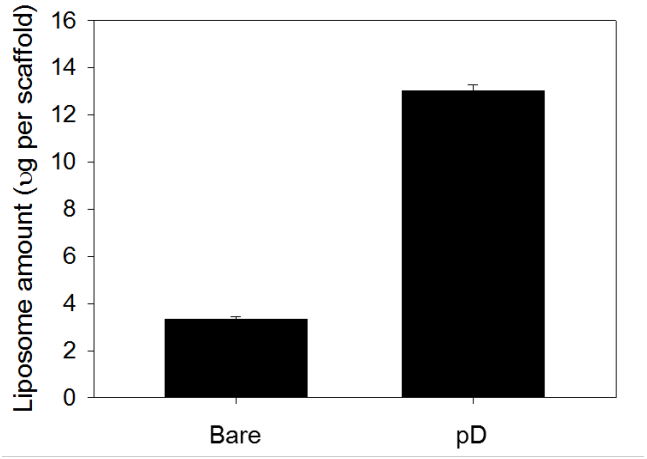

Figure 6. Quantification of immobilized sterosome on surface of PLGA and PLGA-pD via polydopamine for 1 hour. Nile red-loaded sterosome (NR-sterosome) was used as a model liposome. The concentration of NR-sterosome is 1.0 g/L.

Key outcomes and conclusions:

Liposomes loaded on the PLGA scaffold via simple physical absorption were easily affected by surrounding environments and dissociated from the scaffold. We developed a strategy to immobilize liposomes onto the surface of PLGA scaffolds using dopamine intermediates to achieve controlled drug delivery in the defect site.

- Modulate the degradation property of hydrogel scaffolds

We have previously developed a visible blue light-crosslinkable hydrogel system using methacrylated glycol chitosan (MeGC) and riboflavin, an aqueous initiator from natural vitamins. Although the system was useful to localize liposomal nanocarriers to the defect site, it had relatively slow degradation profile which may delay cellular uptake of liposomes and interfere with tissue regeneration. We developed a novel hydrogel system with fine-tuned degradability based on a unique enzyme-substrate pair, lysozyme-chitosan. Lysozyme was modified with photo-crosslinkable moiety for stable conjugation in MeGC hydrogel (**Figure. 7**).

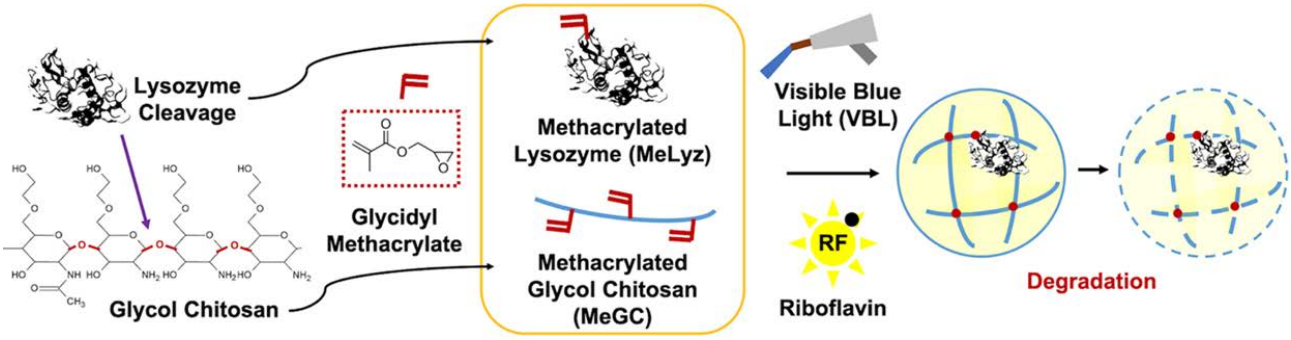

Figure 7. Scheme of degradable hydrogel preparation with MeLyz incorporation. Lysozyme degrades chitosan by cleaving 1,4-β-glycosidic bond in the chitosan backbone. MeGC solution was mixed with methacrylated lysozyme (MeLys) and polymerized with RF initiator under VBL irradiation.

Lysozyme was incorporated in MeGC at various concentrations of 0, 0.1, 1, and 10 mg/ml (MeGC, MeLyz0.1, MeLyz1, and MeLyz10). Degradation profiles of the hydrogels were evaluated by measuring dry weight (**Figure. 8**). The incorporation of lysozyme to chitosan hydrogels accelerated the degradation rate of the crosslinked hydrogels in a dose dependent manner over time. Cryo-freezing and cryo-SEM allows the study of dynamic process of hydrogels degradation with improved ultra-structural preservation and nanometer resolution. The degradation of the three hydrogels was examined by measuring pore size from cryo-SEM micrographs (**Figure. 9a**). At day 0, all three hydrogels yielded comparable pore sizes of 39 ± 14, 37 ± 15, and 38 ± 14 nm respectively for MeGC, MeLyz0.1, and MeLyz1; thereafter increased to 68 ± 27, 86 ± 49, and 409 ± 396 nm respectively, after 10 days (**Figure. 9b**). A clear trend was observed where both the average pore size and size distribution increased as MeLys dose increases for MeLys0.1 and MeLys1. Yet,

MeLys1 hydrogel showed the greatest distribution in pore size with a larger fraction of outliers, reaching up to 1,200 nm at day 10.

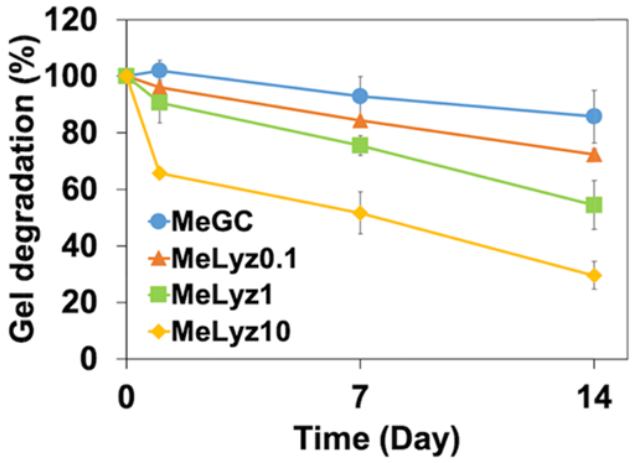

Figure 8. Degradation of MeGC gels functionalized with lysozyme at various concentrations (0, 0.1, 1, and 10 mg/ml). Degradation rate can be controlled by altering amounts of lysozyme conjugation.

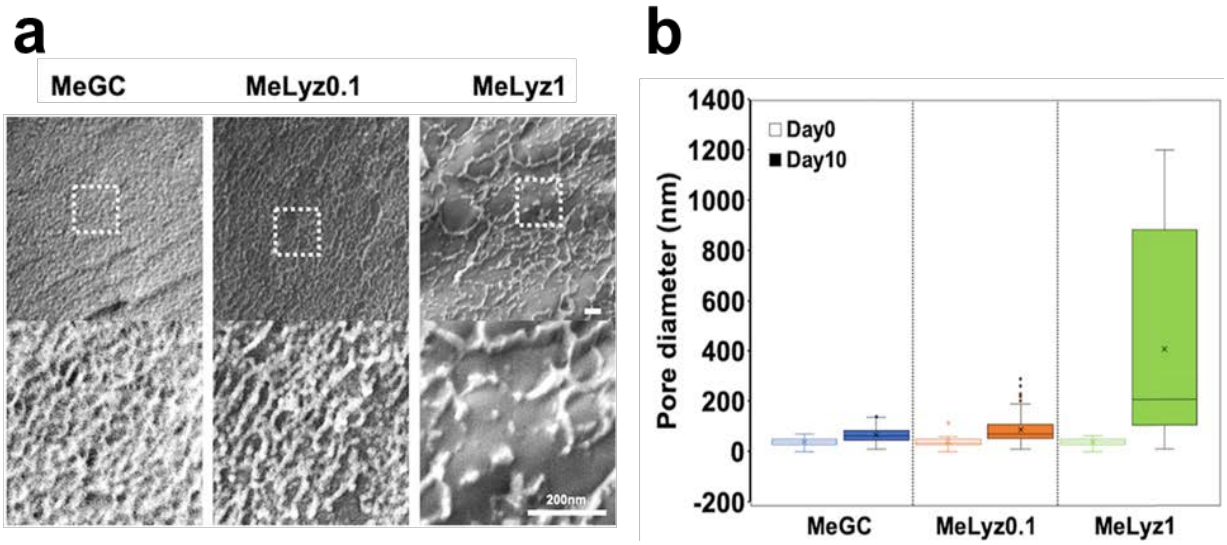

Figure 9. Morphological observation of hydrogels. (a) Cry-SEM micrographs of MeGC gels functionalized with lysozyme at various concentrations (0, 0.1, 1, and 10 mg/ml) 10 days after incubation in PBS. Scale bar = 200 nm.
(b) The box graph of pore diameter from cryo-SEM analysis.

BMSCs were mixed with hydrogel solution (MeGC, MeLyz, and RF) before visible blue light (VBL) crosslinking for cell encapsulation. The viability of BMSCs encapsulated in hydrogels was

observed over two weeks. Fluorescent images and the quantitative analysis demonstrated that cell viability in all hydrogels was above 90% over two weeks (**Figure. 10a**). This indicated that regardless of MeLyz incorporation, hydrogels have biocompatible environments. In spite of the high viability of cells, fluorescent images at day 14 showed larger green-colored areas for MeLyz0.1, and MeLyz1 compared with those for MeGC. This result can be clarified by the cell proliferation result (**Figure. 10b**) pointing out the higher cell proliferation of MeLyz0.1, and MeLyz1 than that of MeGC.

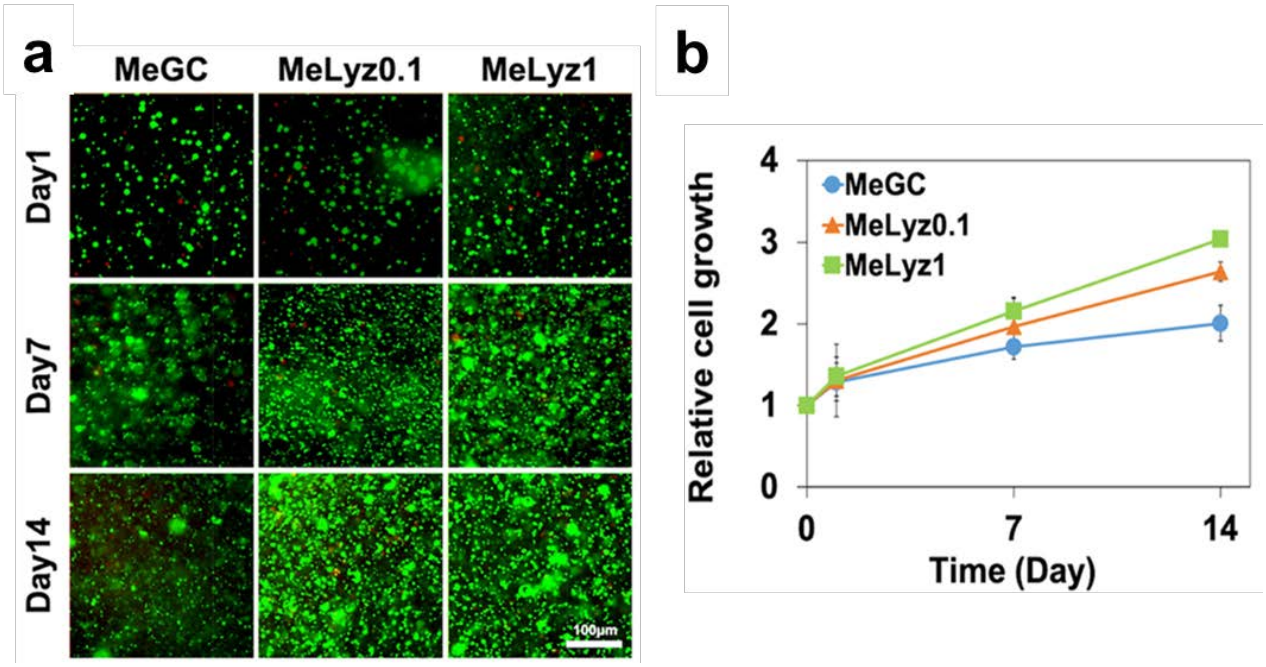

Figure 10. Viability and proliferation of cells in hydrogels. (a) Live/Dead staining images of the BMSCs encapsulated in MeGC, MeLyz0.1, and MeLyz1 for 14 days. (b) Relative cell growth of the BMSCs encapsulated in hydrogels evaluated by the alamarBlue assay.

Osteogenic differentiation of BMSCs encapsulated in the hydrogels was evaluated. The alkaline phosphatase (ALP) expression was assessed by staining and colorimetric assay (**Figure. 11a**). The intensified staining and increment of ALP activity was observed in MeLyz incorporated hydrogels in a dose dependent manner. Next, mineral expression was examined by alizarin red S staining and colorimetric assay (**Figure. 11b**). Similar to the ALP expression, darker staining and elevated calcium production were shown in MeLyz incorporated hydrogels compared with MeGC group.
Lastly, the expression of osteogenic genes such as *Runx2*, *Col1a*, and *Osteocalcin (OCN)* was examined using qRT-PCR analysis (**Figure. 11c**). *Runx2* and *Col1a* were significantly upregulated at day 4 with the incorporation of MeLyz. The expression of late osteogenic gene, *OCN*, was also enhanced at day 21 in MeLyz incorporated hydrogels. All three osteogenic genes were highly produced in a MeLyz dose dependent manner.

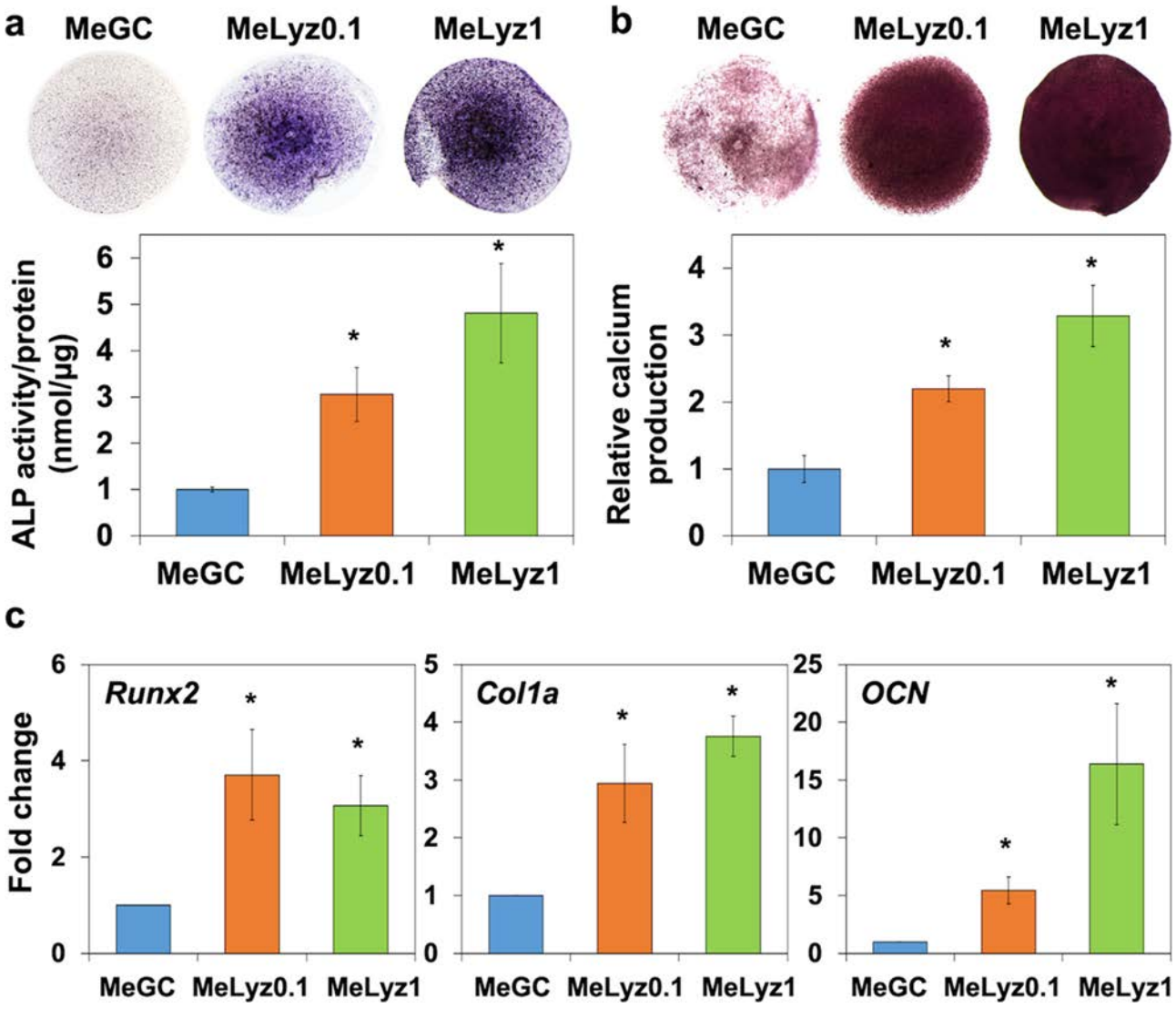

Figure 11. *In vitro* osteogenesis of cells in hydrogels. (a) ALP staining images of BMSCs in hydrogels and quantified ALP activity normalized by total protein production at day 4. b) Alizarin red S staining images of BMSCs in hydrogels and quantified calcium production at day 21. c) Osteogenic gene expression of BMSCs analyzed by qRT- PCR at day 4 (*Runx2*, *Col1a*) and day 21 (*OCN*)

Key outcomes and conclusions:

Although we were able to localize liposomal nanocarriers to the defect site using MeGC hydrogels in our previous study, slow degradation of the hydrogel may delay uptake of liposomes by cells and tissue regeneration. We successfully modulated the degradation behavior of MeGC hydrogels by incorporating the hydrogel-specific enzyme, lysozyme. This system will be useful in delivery of liposome nanoparticles in conjunction with PLGA scaffolds.

What opportunities for training and professional development has the project provided?

This project provided a number of opportunities for Dr. Chung-Sung Lee and Dr. Xiao Zhang, a postdoctoral researcher, to learn various drug delivery techniques. They also acquired in-depth knowledge of signaling molecules and mechanisms involved in osteogenic differentiation. Novel findings from the project have been presented at the following conferences:

1. Annual Micro-and Nanotechnologies for Medicine Workshop - Emerging Frontiers and Applications, Los Angeles, CA, July 8-12, 2019.
2. UCLA Advanced Prosthodontics Science Day, Los Angeles, California, June 14, 2019

How were the results disseminated to communities of interest?

We have disseminated our novel findings by presenting our work and interacting with other investigators and leaders in the field at the following conferences:

1. Lee M. Nanomaterials for Bone Regeneration. Orthopaedic Research Society (ORS), Southern California Regional Symposium, Los Angeles, California, October, 2018
2. Lee M. Orthobiologics: Complementary Osteoinductive Therapy. BK21 Plus International Symposium on Innovative Biomaterials for Future Therapy, Seoul, Korea, May, 2019
3. Lee CS, Lee M. A hybrid scaffold embedded with smoothened agonist non-phospholipid liposome for bone healing. Annual Micro-and Nanotechnologies for Medicine Workshop - Emerging Frontiers and Applications, Los Angeles, CA, July 8-12, 2019.

What do you plan to do during the next reporting period to accomplish the goals?

We demonstrated that liposomes formulated with SA and Oxy enhanced osteogenic differentiation of MSCs. During the next year, we will evaluate the ability of SA/Oxy nanoparticles to deliver small molecule SAG and enhance Hedgehog (Hh) signaling in MSCs. *In vivo* efficacy of SAG-loaded liposomal nanoparticles will be tested in a critical size calvarial defect.

Although our SA/Oxy nanoparticles have been delivered to the defect site using a photocrosslinkable chitosan hydrogel system in our previous study, they have not been tested on PLGA scaffolds. During the next year, we will continue to develop a strategy to immobilize liposomes onto the surface of PLGA scaffolds and evaluate their activity on the PLGA scaffolds. Alternatively, we will enhance the binding affinity of liposomes with apatite-coated PLGA via nanoparticle modification with bisphosphonate. Bisphosphonate such as alendronate has been widely used to develop nanoparticles targeting bone matrix.

SA/Oxy nanoparticles were found to be cytotoxic at higher concentration over 10 ug/ml due to its strong cationic nature. Although positive charge seems to improve drug delivery efficacy, cationic nanoparticles can disrupt plasma-membrane integrity and cause mitochondrial and lysosomal damage. During the next year, we will fabricate sterosomes using negatively charged mixtures of palmitic acid with Oxy and evaluate its bioactivity in comparison to SA/Oxy nanoparticles.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

This study developed a novel class of liposomes composed of non-phospholipid molecules formulated with single-chain amphiphiles and high content of sterols (sterosomes). The high sterol content in our sterosomes induces well-ordered lipid bilayer chains with very limited permeability and significantly increased nanoparticle stability compared to conventional phospholipid. This system can be advantageous for delivery of small molecular drugs or other therapeutic genes. Moreover, oxysterols used in this study are interesting sterol molecules for applications targeting damaged bone treatment. Oxysterols have been shown to induce osteogenic differentiation of MSCs and increase anabolic mineralization by regulating Hh signaling. The additional knowledge gained from this study may suggest nanocarrier design strategies loading bioactive agents into functional non-phospholipid bilayers to improve clinical efficacy of current therapeutic agents.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

6. PRODUCTS

- **Publications, conference papers, and presentations**

Journal publications.

1. Kim S, Cui Z, Koo B, Zheng J, Aghaloo T, Lee M. Chitosan-Lysozyme Conjugates for Enzyme-Triggered Hydrogel Degradation in Tissue Engineering Applications. *ACS Applied Materials & Interfaces* 10(48):41138-41145, 2018. Federal support acknowledged.

2. Cui Z, Kim S, Baljon J, Wu B, Aghaloo T, Lee M. Microporous chitosan-montmorillonite nanocomposite hydrogel for bone tissue engineering. Under review. Federal support acknowledged

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

1. Lee M. Nanomaterials for Bone Regeneration. Orthopaedic Research Society (ORS), Southern California Regional Symposium, Los Angeles, California, October, 2018
2. Lee M. Orthobiologics: Complementary Osteoinductive Therapy. BK21 Plus International Symposium on Innovative Biomaterials for Future Therapy, Seoul, Korea, May, 2019
3. Lee CS, Lee M. A hybrid scaffold embedded with smoothened agonist non-phospholipid liposome for bone healing. Annual Micro-and Nanotechnologies for Medicine Workshop - Emerging Frontiers and Applications, Los Angeles, CA, July 8-12, 2019.

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

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

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Aaron James
Project Role:	Initiating PI
Researcher Identifier	https://orcid.org/0000-0002-2002-622X
Nearest person month worked:	1
Contribution to Project:	Dr. James is responsible for the overall design and conduct of the project, oversight of all research activities, budget management, publication writing and scientific report preparation.
Funding Support:	NIH, DoD, American Cancer Society, MTF Biologics, Maryland Stem Cell Research Fund

Name:	Yongxing Gao
Project Role:	Senior Research Specialist
Researcher Identifier	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Gao is responsible for animal care and maintenance during the current funding period.
Funding Support:	NIH / DOD /American Cancer Society

Name:	Sarah Miller
Project Role:	Research Technologist
Researcher Identifier	N/A
Nearest person month worked:	10
Contribution to Project:	Ms. Miller is responsible for animal care, histologic and histomorphometric analyses.
Funding Support:	N/A

Name:	Ching-Yun Hsu
Project Role:	Postdoctoral Researcher
Researcher Identifier	N/A

Nearest person month worked:	10
Contribution to Project:	Dr. Hsu is responsible for radiographic imaging and animal care related to the current project.
Funding Support:	N/A

Name:	Takashi Sono
Project Role:	Postdoctoral Researcher
Researcher Identifier	https://orcid.org/0000-0001-5599-0185
Nearest person month worked:	2
Contribution to Project:	Dr. Sono is responsible for surgical procedures related to the current project.
Funding Support:	DOD/American Cancer Society

Name:	Jiajia Xu
Project Role:	Postdoctoral Researcher
Researcher Identifier	https://orcid.org/0000-0002-6084-2029
Nearest person month worked:	2
Contribution to Project:	Dr. Xu is responsible for post-mortem histologic / immunohistochemical analyses related to the current project.
Funding Support:	DOD/American Cancer Society

Name:	Min Lee
Project Role:	Partnering PI
Researcher Identifier	orcid.org/0000-0003-2813-2091
Nearest person month worked:	2
Contribution to Project:	Dr. Lee is responsible for the overall design and conduct of the project within UCLA, oversight of all research activities, budget management, publication writing and scientific report preparation.
Funding Support:	NIH/NIDCR

Name:	Chung-Sung Lee

Project Role:	Postdoctoral Researcher
Researcher Identifier	orcid.org/0000-0001-5813-6056
Nearest person month worked:	8
Contribution to Project:	Dr. Lee has fabricated SA/oxysterol drug delivery systems, and also performed material characterization and data analysis
Funding Support:	N/A

Name:	Xiao Zhang
Project Role:	Postdoctoral Researcher
Researcher Identifier	orcid.org/0000-0002-0242-1939
Nearest person month worked:	8
Contribution to Project:	Dr. Zhang has performed the daily research and participated in the bioactivity test of drug-loaded liposomes as well as molecular biology analyses.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Aaron James:

Previously active grant that has closed:

Title: Regional specification of bone-associated perivascular MSC
Time commitment: PI (0.6 calendar months)
Supporting agency: MSCRF Discovery Award
Contracting/Grants Officer: Dan Gincel, 7021 Columbia Gateway Drive, Suite 200, Columbia, MD 21046
Performance Period: 07/01/2017 – 06/31/2019
Level of funding: $300,000
Brief description of overall goals: To examine the regional specification of periosteal and bone marrow pericytes, and compare their differential stem/progenitor cell content.
Specific Aims:
AIM 1: Define the regional specification of periosteum- and bone marrow-derived human pericytes.
AIM 2: Determine how periosteum- or bone marrow-origin impacts the regenerative phenotype of human pericytes.

Previously pending grants that are now active:

Title: Endogenous and Exogenous Pericytes in the Pathobiology and Treatment of Osteoarthritis
Time commitment: PI (0.6 calendar months)
Supporting agency: Department of Defense
Contracting/Grants Office: Kevin R Moore, US Army Medical Research Acquisition Activity, 1120 Fort Detrick, Frederick, MD 21702
Performance Period: 07/01/18 – 12/31/2019
Level of funding: $197,056
Brief description of overall goals: To evaluate the location and immunomodulatory function of pericytes within the osteoarthritic afflicted synovium.
Specific Aims:
AIM 1: Develop and evaluate a post-traumatic osteoarthritis model within pericyte reporter mice
AIM 2: Evaluate the therapeutic potential of intra-articular pericyte delivery in mouse post-traumatic osteoarthritis.

Title: Dkk1 Antagonism for Stromal Vascular Fraction (SVF) Mediated Bone Repair
Time commitment: PI (1.2 calendar months)
Supporting agency: Department of Defense
Contracting/Grants Office: US Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick, MD 21702-5014
Performance Period: 09/30/2018-09/29/2023
Level of funding: $974,170
Brief description of overall goals: To examine the improvement in stromal vascular fraction mediated bone formation with the use of Anti-DKK1.
Specific Aims:
AIM 1: Determine how DKK1 antagonism impacts human ASC lineage commitment in vitro.
AIM 2: Validate the combination of anti-DKK1 and SVF in femoral bone defect regeneration.
AIM 3: Challenge anti-DKK1 + SVF therapy in oteoporotic lumbar spinal fusion.

Title: Role of sensory nerves in cranial bone formation and repair
Time commitment: Co-PI (0.6 calendar months)
Supporting agency: NIH / NIDCR
Contracting/Grants Officer: Guo H. Zhang, 6701 Democracy Boulevard, Room 674, Bethesda, MD 20892
Performance Period: 07/01/2018 – 06/31/2020
Level of funding: $275,000
Brief description of overall goals: To examine the role of sensory nerves in calvarial defect healing.
Specific Aims:
AIM 1: Map sensory nerve innervation and NGF expression in cranial bone repair.
AIM 2: Determine the requirement of NGF-dependent TrkA sensory nerves in cranial bone repair.

Min Lee:

Previously active grant that has closed:

Title: Combined effects of noggin suppression and Nell-1 on bone regeneration
Time commitment: PI (3.6 calendar months)
Supporting agency: NIH/NIAMS, R01 AR060213
Contracting/Grants Officer: Fei Wang, NIAMS 6701 Democracy Blvd, Ste 800 Bethesda, MD 20892-472
Performance Period: 7/25/11 – 6/30/18 (NCE)
Level of funding: $1,708,245
Project Goals: To examine the synergism between noggin and Nell-1.
Specific Aims/Tasks: 1) To enhance bone regeneration via Noggin suppression + Nell-1; 1.1) To enhance MSC osteogenesis with Noggin suppression and Nell-1 stimulation, 1.2) To enhance long bone regeneration *in vivo* with Noggin suppression and Nell-1 stimulation, 2) To enhance bone regeneration via controlled delivery of Noggin-siRNA + Nell-1; 2.1) Development of scaffolds for controlled delivery of siRNA and Nell-1, 2.2) To enhance long bone regeneration *in vivo* via controlled delivery of Noggin-siRNA + Nell-1
Overlap: None

Previously pending grant that is now active:

Title: Tribbles homolog 3 and BMP-2 induced bone formation
Time commitment: PI (3.6 calendar months)
Supporting agency: NIH/NIDCR, R01 DE027332
Contracting/Grants Officer: Jason Wan, NIDCR 6701 Democracy Blvd. Rm 620, Mail Stop 4878 Bethesda, MD 20892-4878
Performance Period: 6/4/18 – 5/31/23
Level of funding: $1,925,000
Project Goals: To investigate whether overexpression of Trb3 and simultaneous abrogation of BMP antagonism can enhance BMP-induced bone formation quality
Specific Aims/Tasks: 1) To determine efficacy of Trb3 to improve BMP-2 mediated bone regeneration, 2) To determine efficacy of Trb3 combined with noggin suppression to enhance osteogenesis, 3) To enhance bone regeneration with controlled delivery of Trb3 DNA + noggin siRNA
Overlap: None

What other organizations were involved as partners?

University of California, Los Angeles
10920 Wilshire Blvd., Ste. 600
Los Angeles, CA 90024

All scaffold fabrication and validation is performed at the partnering PI site.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Independent reports were submitted from the Initiating PI and Partnering PI.

QUAD CHARTS:

Not applicable

9. APPENDICES:

Award Chart

PR170115: Development of Smoothened Agonist Non-Phospholipid Liposomal Nanoparticles for Bone Repair

PI: Aaron W. James, Johns Hopkins University, MD **Budget:** $1,357,063

Topic Area: Nanomaterials for bone regeneration **Mechanism:** W81XWH-17-PRMRP-IIRA

Research Area(s): 0802, 0803, 0817, 0822 Award Status: 15 July 2018 – 14 July 2019

Study Goals:

To develop a nanoparticle delivered small molecule for faster, safer, and more efficacious bone repair than currently available treatment strategies.

Specific Aims:

Aim 1: Optimize SAG-loaded liposomal nanoparticles for mouse calvarial defect repair.

Aim 2: Determine the safety of SAG-loaded liposomal nanoparticles for mouse calvarial defect repair.

Key Accomplishments and Outcomes:

Publications:

1. Kim S, Cui Z, Koo B, Zheng J, Aghaloo T, Lee M. Chitosan-Lysozyme Conjugates for Enzyme-Triggered Hydrogel Degradation in Tissue Engineering Applications. ACS Applied Materials & Interfaces 10(48):41138-41145, 2018.

Patents: none to date
Funding Obtained: none to date