Duquesne University

Annual Progress Report: 2007 Formula Grant

Reporting Period

July 1, 2009 – June 30, 2010

Formula Grant Overview

The Duquesne University received $84,549 in formula funds for the grant award period January 1, 2008 through December 31, 2010. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Expanded Health Belief Model & Adherence to Mammography Screening in Women - The purposes of this study are to describe and examine the variables associated with the Expanded Health Belief Model (EHBM) and adherence to mammography screening in women with rheumatoid arthritis (RA), and to explore the extent to which the EHBM variables can predict adherence to mammography screening in women with RA compared to women without RA.

Anticipated Duration of Project

1/1/2008 - 12/31/2010

Project Overview

The broad objective of this project is to gain a better understanding of the Expanded Health Belief Model (EHBM) variables and their ability to predict adherence to mammography screening in women with rheumatoid arthritis (RA) and without RA. The specific aims of this study are to: 1) describe the EHBM variables including perceived susceptibility and severity, perceived benefits and barriers, cues to action, self-efficacy and adherence to mammography screening in women with RA; 2) explore potential predictors of adherence to mammography screening in women with RA; and, 3) compare predictors of adherence to mammography screening in women with RA to women without RA.

Design**:** A descriptive, correlation design will be used in this project to examine the EHBM variables including perceived susceptibility and severity, perceived benefits and barriers, cues to action, and self-efficacy and to gain a better understanding of potential predictors of adherence to mammography screening in a group of women with RA compared to a group of women without RA.

Sample and Settings**:** Women with RA and women without RA will be recruited for this project from two different settings within the Western Pennsylvania Health System. Results of a power

analysis using a moderate effect size of .40 indicated that a total of 300 women or 150 women per group will be needed to achieve a power of .80 and an alpha of .05 using a two tailed test of significance.

Women with RA will be recruited from a rheumatology practice and women without RA will be recruited from a small community hospital Emergency Department (ED). Only women between 40 and 70 years of age with a diagnosis of RA, who are fluent in English and have at least an 8th grade education, and do not have a current diagnosis of breast cancer will be included in the RA group. Women in the comparison group will only be included in the project if they are 40 to 70 years old, have never been diagnosed with RA or breast cancer, are fluent in English, and have at least an 8th grade education.

Instruments: Several questionnaires will be used to collect data including the Demographic Data Form, The Breast Cancer Knowledge Test (BCKT), Benefits and Barriers Mammography Screening Test-Champion, the Short Form-36 (SF-36), the Beck Depression Inventory, 2nd edition (BDI-II), and the Mammography Screening Self-Efficacy Scale (MSSS).

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Expected Research Outcomes and Benefits

Barriers to mammography screening are present in women in the general population as well as in women with disabilities such as rheumatoid arthritis (RA). Women with RA have specific disease related variables such as physical limitation and depression that may prevent adherence to mammography screening. There may however be other variables that prevent mammography screening in women with RA. Although components of the EHBM are well established in healthy women, no studies to date have examined the extent to which RA related variables contribute to underutilization of mammography screening in women with RA.

An estimated 178,480 women are expected to be diagnosed with breast cancer in 2007. Recommended screening for breast cancer includes an annual mammogram in women age 40 and above (American Cancer Society, 2005). RA affects 1% of the United States, or 2.1 million Americans. It can affect anyone at any age, but 70% of people with RA are women. Efforts are needed to improve the quality of care for RA patients and to increase physician awareness of co-morbid diseases among patients with RA and other chronic diseases. In one group of women

analyzed with RA, only 32% of the sample received a mammogram during the past year (Kremers et al. 2003).

This research project will aid in identifying specific factors that may contribute to the low mammography screening rate in women with RA. It is necessary to know the extent to which EHBM variables predict adherence to mammography screening in women with RA in order to develop interventions that will promote adherence to mammography screening and thus, help prevent breast cancer in this population of women. This project is significant to nursing and will provide valuable insight into the delivery of health care services to reduce heath risks and transfer research advances to community use, with the prevention and reduction of disease.

Summary of Research Completed

Expanded Health Belief Model & Adherence to Mammography Screening in Women
Design: A descriptive, correlation design was used in this study to examine the EHBM variables including perceived susceptibility and severity, perceived benefits and barriers, cues to action, and self-efficacy and to gain a better understanding of potential predictors of adherence to mammography screening in a group of women with RA compared to a group of women without RA.

Sample and Settings: Women with RA and women without RA were recruited for this study from two different settings within the Western Pennsylvania Health System. Women with RA were recruited from a rheumatology practice of a physician who has agreed to serve as a co-investigator in this study. Women without RA who presented at the Emergency Department (ED) with a non-urgent problem were recruited at a small community hospital located in Southwestern Pennsylvania. The Medical Director of the ED also was a consultant for this study.

Only women between 40 and 70 years of age with a diagnosis of RA verified by a rheumatologist, who are fluent in English and have at least an 8th grade education, and do not have a current diagnosis of breast cancer were included in the RA group. Women in the comparison group were only included in the study if they were 40 to 70 years old, have never been diagnosed with RA, breast cancer, or depression, are fluent in English, and have at least an 8th grade education.

Recruitment was completed in January of 2010. At that time, initial analysis was completed. Future analysis will include a logistic regression analysis. In addition, a manuscript has been started with revisions being completed. Three abstracts also were submitted for national presentations, and two have been accepted with the other pending a review.

Data Analysis and Interpretation:

For continuous variables, distributions were evaluated. Age, efficacy, knowledge, curability, barriers, and education were found not to be normally distributed. Benefits however, was normally distributed/ Spearman's correlation coefficients were used to evaluate the relationship between efficacy, knowledge, curability, barriers, BDI, and education. The highest correlation

coefficients were for barriers and efficacy (-0.44), education and knowledge (0.25), and barriers and BDI (0.21).

Demographics and characteristics were compared between RA and ED groups using t-tests for continuous approximately normally distributed variables, Wilcoxon rank-sum tests for variables that were not normally distributed, and chi-square tests for categorical variables. Predictors of mammography were examined for the combined group, RA and ED groups using univariate and multivariate logistic regression models. Variables with p-values less than .2 in univariate models for either RA or ED group were included in the multivariate models.

Results:

The mean age of the combined groups is 53 years, and the RA group is older that the ED group (53 vs. 52 years, p=.018). Of the total group, 95% are White and 5% African American; 50% reported more than 12 years of schooling, and 94% have health insurance. A higher percentage of the RA group had health insurance (99% vs. 90%, p=.026). The mammography rate was similar between groups with 62% reporting having a mammogram in the past year. Depression was also similar between groups with 73% being minimally depressed. Benefits, efficacy, curability, and barriers were similar between the groups. Knowledge was higher for the RA group (median=9 vs. 8; p=.038).

Variables Associated with Mammography in Past Year

For the overall group, based on univariate logistic regression models, physician recommendation, having health insurance, greater benefits, and greater efficacy were associated with an increased likelihood of having a mammography in the past year. Having greater barriers was associated with a decreased likelihood of having a mammogram in the past year. Results were similar when stratified by RA and ED groups with the exception that within RA patients: an increased BDI score was associated with a decreased likelihood of mammogram in past year, and increased benefits was not statistically significantly associated with mammography in past year.

Table 1. Characteristics of Sample

	Total (n=160)	**RA (n=73)**	**ED (n=87)**	**P-value**
Median Age, (IQR)	53(8.1)	55(7.8)	52(8.1)	.018
Race (5) White African-American	 95.4 4.5	 98.6 1.4	 92.7 7.3	 .08
Education >12 Years (%)	50.3	52.8	48.2	.6
Health Insurance (%)	94.1	98.6	90.1	**.026**
Physician Recommendation (%)	83.0	88.9	77.8	.07
Mammogram in the Last Year (%)	62.3	68.5	56.8	.13
Beck Depression Inventory (%) Minimal (0-13) Mild-severe (>13)	 73.4 26.6	 76.4 23.6	 70.9 29.1	 .4
Mean Benefits (SD)	26.1 (4.1)	26.6 (4.0)	26.4 (4.1)	.8
Median Efficacy (IQR)	254 (188-310)	269 (194-312)	236 (186-306)	.2
Median Knowledge (IQR)	9 (8-10)	9 (8-10)	8 (7-10)	**.038**
Median Curability (IQR)	7 (6-8)	7 (6-8)	7 (6-8)	.5
Median Barriers (IQR)	10 (8-13)	10 (8-13)	10 (8-12)	.9

Table 2: Univariate Logistic Regression Models for Mammography in Past Year

	TOTAL OR	**TOTAL P-value**	**RA OR**	**RA P-value**	**ED OR**	**ED P-value**
RA	1.65	.14				
Age	1.04	.08	1.02	.5	1.04	.17
Race	.80	.7			.74	.7
Education 12 + yrs	1.14	.7	1.7	.28	.79	.6
Physician Recommendation	20.2	**<.001**			10.8	**.001**
Insurance	6.4	**.02**			4.6	.075
BDI	1.00	.7	.93	**.047**	1.00	.8
Benefits	1.12	**.01**	1.07	.3	1.17	**.013**
Efficacy	1.01	**<.001**	1.01	**.008**	1.01	**.003**
Knowledge	.96	.7	.98	.9	.90	.4
Curability	.89	.4	1.03	.9	.77	.24
Barriers	.82	**<.001**	.84	**.013**	.79	**.003**

Research Project 2: Project Title and Purpose

Development of a Bone-Regenerative Scaffold - The goal of this project is to develop a novel therapy to aid in the formation of bone by first developing a ceramic scaffold on which stem cells will attach, grow, and form bone; and by second attaching to the scaffold a hormone (melatonin) that has the ability to enhance the formation and function of these bone-forming stem cells.

Anticipated Duration of Project

1/1/2008 - 12/31/2010

Project Overview

The goal of this project is to develop a novel therapy to aid in the formation of bone by way of the following Specific Aims: 1) To develop a calcium aluminate ceramic scaffold that facilitates bone formation that is non-toxic and 2) To determine the efficacy of this calcium aluminate ceramic scaffold on forming bone in a rat.

The hypothesis that underlies this proposal is that a novel use of a biomaterial called calcium aluminate along with melatonin will enhance the formation of bone in a rat model.

For Aim 1, new technologies and products combined with novel drug therapies are needed to treat injuries and bony defects in cranial and maxillofacial tissues, as well as critical fractures and bone/joint replacement. Ideal treatments would provide off-the-shelf materials that serve as strong, biodegradable scaffolds that have the ability to contain biological factors such as hemostatic agents, analgesics, antibiotics, drugs, hormones, and/or cells to stabilize, protect, and promote tissue repair. Here, we have investigated a potential biomaterial, calcium aluminates (CA), which has a number of desirable properties including high mechanical strength, controllable porosity, ease of formation, and room temperature strength development that gives this material physical advantages over currently available bone graft and implant technologies. In addition, typical chemical moieties present in the various forms of CA offer attractive ionic and/or covalent binding sites for attachment of biological factors (i.e., melatonin) that can enhance tissue formation and repair.

Regarding Aim 2, preliminary data using a mixed phase material of $Ca_3(Al(OH)_6)_2$, $Ca(AlO_2)_2$, and $CaAl_4O_7$, is effective at supporting cell adhesion, proliferation and differentiation of adult mesenchymal stem cells into osteoblasts *in vitro*. In a rat skull defect (8mm) study, the calcium aluminate material implanted in the defect proved to be non-toxic over the course of four weeks and effectively filled the defect better than the control or commercial material, Vitoss®. We propose to test a novel drug therapy including the development of a calcium aluminate ceramic scaffold to induce bone formation in the rat and will be adding novel biological agents (i.e., platelet-rich plasma and melatonin) to see if they improve bone formation in the rat.

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Expected Research Outcomes and Benefits

Americans today enjoy greater longevity (77.6 years) than any other generation and are undergoing implanted prosthetic joint and dental implant procedures on a routine basis. Also, many of our injured soldiers lose limbs and require extensive prosthetic surgery. Often times these patients require bone grafting procedures to accommodate placement and retention of these prosthetic joints, limbs, and teeth. The use of bone grafts often necessitates that additional time is allotted for complete bone formation and frequently the final results are less than what was expected. Over one million bone grafting procedures are completed each year in the United States alone. Autogenous bone grafts are obtained from a second surgical site or allograft bone grafts are obtained from cadavers. Each of these sources presents with multiple problems. Autogenous grafts can lead to morbidities (pain, infection, functional restrictions) associated with the donor surgical sites. Allograft bone grafts present with the concern of infectious disease transmission and possible irregularities in processing donor bone grafts that lead to recalls of materials that may have already been implanted in the patient. Because of these real-world concerns the medical and dental professions plus patients would benefit greatly if a synthetic graft material were available that would negate the need for using autogenous grafts or cadaver-derived bone grafts. It would be beneficial if the synthetic material could also be supplemented with pharmacological agents [melatonin and Platelet-Rich Plasma (PRP)] that would reduce both healing time and complications plus provide greater predictability. Additionally, it would be helpful if the grafting material could be a pliable material that would harden shortly after placement and therefore assuring that the graft stays in the intended location when the clinician closes the surgical site. All of these attributes would translate into a significant benefit for the professions and patients.

Summary of Research Completed

The <u>hypothesis</u> that underlies this proposal is that a novel use of a biomaterial called calcium aluminate along with melatonin will enhance the formation of bone in a rat model.
The goal of this project is to develop a novel therapy to aid in the formation of bone by way of the following Specific Aims:

Specific Aim 1: To develop a calcium aluminate ceramic scaffold that facilitates bone formation that is non-toxic.

In addition to the cell viability assays, which showed calcium aluminate scaffolds functionalized with melatonin (CA-mel) show preference for osteoblast adherence and proliferation compared to unmodified calcium aluminate (CA) scaffolds alone after 7 days in culture; chick chorioallantoic membrane (CAM) assays were also used to determine the toxicity of modified and unmodified scaffolds. The CAM assay is a common method for studying the tissue response to biomaterials and is predictive of the tissue response in a mammalian model. Additionally, this model can be used to investigate angiogenesis and vascular injury. Thus, it provides an adequate analysis of inflammation and toxicity for the biomaterials utilized in this study. In this study, the modified and unmodified CA scaffolds were placed on the chick embryo and incubated for 24, 48 or 72 hours. Embryo development and vascularization was studied by visual observation and brightfield microscopy as shown in Figure 1. Vessel growth occurred around all scaffolds and all embryos survived the testing indicating that the material is biocompatible. Further, there were no signs of vascular injury (i.e., hemorrhage, lysis, and/or coagulation).

Specific Aim 2: To determine the efficacy of this calcium aluminate ceramic scaffold on forming bone in a rat.

Melatonin enhances the differentiation of hAMSCs into osteoblasts, as evidenced by an increase in alkaline phosphatase activity. These studies substantiate claims that melatonin may accelerate the synthesis and mineralization of the osteoid matrix and aid in bone formation. The second aim of this proposal was to investigate the effects of calcium aluminate functionalized with melatonin on bone regeneration in a rodent model of calvaria (i.e., skull cap) defects. It is believed that calvarial defects will better reflect the bone regenerating capacity of the unmodified and modified calcium aluminate scaffolds because there is thin flat bone peripherally with no bone inferior or superior to the defect. The typical long cortical bone defect has larger quantities of peripheral bone and bone inferior to the defect, which accommodates bone formation. Ovariectomized Sprague-Dawley rats were chosen for this proposal because they are representative of the post-menopausal female population that is likely to seek dental implants and prosthetic joint replacements. The defect size reported below is the minimum sized defect that will not heal spontaneously during the lifetime of the animal. As already reported, 5mmx5 mm osteotomies were created on superior aspect of frontal bone in ovariectomized Sprague-Dawley rats (250-300 grams) using an ultrasonic Piezotome™ handpiece.The Piezotome™ allowed for cutting of the frontal bone without affecting the underlying cerebral hemispheres. Following the creation of the calvarial defects, rats were implanted with either the calcium aluminate (CA) scaffold or the calcium aluminate scaffold covalently attached melatonin (CA-Mel). As already reported, the calcium aluminate scaffold material was optimized for pore size, bulk density, modulus of rupture, and cold crushing strength.

Bone regeneration at the surgical site was assessed by histology and double-fluorochrome labeling at three and six months (Figure 2). Double-fluorochrome labeling was achieved with separate subcutaneous injections of tetracycline and calcein given seven days apart. A caveat to this technique is that these fluorochromes are only incorporated into sites of *active* bone remodeling. The presence of each incorporated fluorochrome can be detected with fluorescence

microscopy. Thus, qualitative information can be gathered about the degree and rate of osteoid synthesis and mineralization.

Histological assessments for animals undergoing osteotomy and scaffold implantation were completed at three and six months. Skulls were prepared after necropsy and sent to the University of Alabama at Birmingham, Center of Metabolic Bone Disease for sectioning and staining. Five-micrometer ground sections were examined for the presence of incorporated fluorochromes and later stained with methylene blue/basic fuschin for histological assessment (Figure 3). The stain used for the histological assessment highlights cellular proteins (e.g., bone, collagen, and connective tissue; pink/purple) and DNA (e.g. cell nucleus; blue/dark purple). A methylene blue/basic fuchsin stain was used to examine general histology of the defect site and scaffold circumference for signs of scaffold integration, tissue infiltration into scaffold pores, and osteoid synthesis along the scaffold surface. Analysis of stained sections indicates that implanted scaffolds provide a favorable surface for the growth and incorporation of cells and connective tissues. Furthermore, because the fluorescence around and through the scaffolds overlaps the cells and proteins depicted by the histological stain, the fluorescence noted in these micrographs, and later quantified, is not a result of artifacts from the staining or visualization procedures. In addition, histological assessments may support the biodegradation of the calcium aluminate scaffold along the implant border (e.g., note the irregular border of the calcium aluminate compared to the smooth surfaces).

Skull/implant sections from each experimental group were visualized with fluorescence microscopy to assess the incorporation of the injected fluorochromes as an indication of active bone remodeling. Quantitative assessments were made from double-labeled sections based on the percentage of scaffold fluorescence and the number of fluorescent regions. Scaffold fluorescence as a percentage of scaffold area and number of fluorescent regions were quantified and statistically compared between groups to determine the impact of treatments on bone remodeling at the defect site (Figure 3). The degree of scaffold fluorescence was significantly greater for the CA-Mel group at three months compared to the CA alone groups. Likewise, the number of fluorescent regions was found to be significantly greater for the CA-Mel group at six months when compared to CA alone. These data show that the CA-Mel implant significantly impacts the degree of osteoid tissue mineralization and bone formation in this model.

Additional photomicrographs from 3- and 6-month animals are included in Figure 4. Closer examination of the implant site indicates scaffold integration, tissue infiltration into scaffold pores, and osteoid synthesis along the scaffold surface (Figure 4). It appears that many of the cells that were present around the implanted scaffolds at 3 months have now been largely replaced by bone matrix tissues (e.g., parallel bands of collagen or lamellar bone attachments). Thus, the cells noted in the 3 month photomicrographs are likely bone-derived cells such as osteoblasts and osteoclasts that functioned to synthesize and mineralize the bone matrix over time, as other cell types (i.e., fibroblasts) would not contribute to bone remodeling. This is further supported by the preference of the calcium aluminate material for osteoblasts versus fibroblasts (data in previous report; not shown here) and the qualitative and quantitative results from the double-labeled sections (Figures 2,3).

Overall, based on the results of Aim 2, 1) the implanted scaffolds support the growth and expansion of cells and tissues derived from the defect site and the surrounding bone, 2) these

cells later contribute the synthesis and mineralization of osteoid tissue, 3) the quantified fluorescence is a reliable predication of bone remodeling activity for the experimental groups, and 4) if the calcium aluminate is degrading at the implant site, the rate of biodegradability is matched by a similar rate of bone fill.

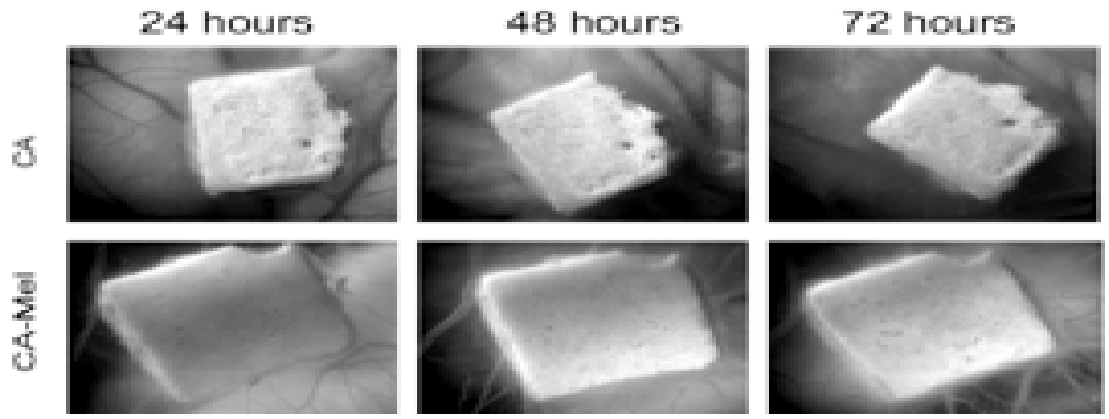

Figure 1. Chorioallantoic membrane (CAM) assays confirm unmodified and modified calcium aluminate scaffolds are nontoxic.

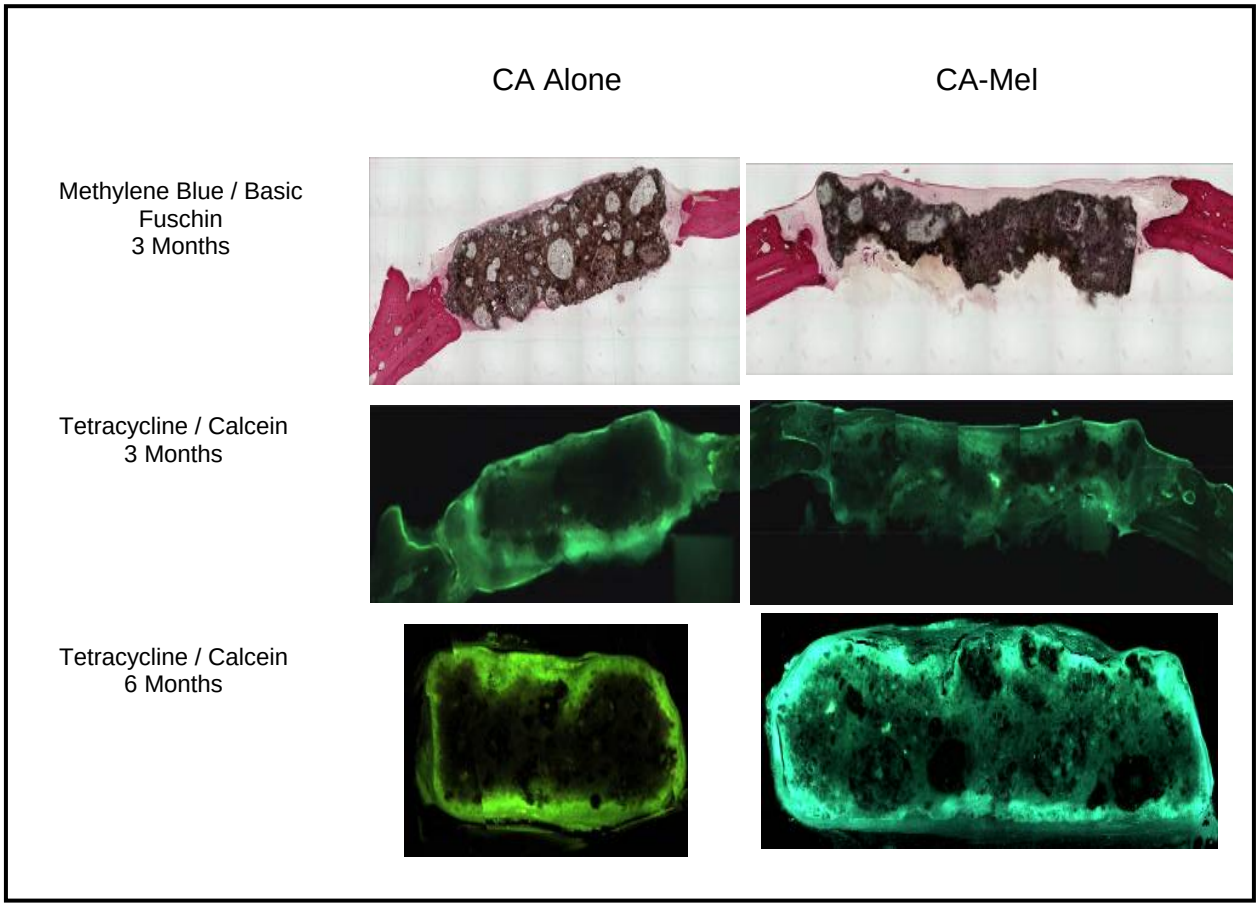

Figure 2. Scaffold histology and double-fluorochrome labeling at three and six months. Representative micrographs of experimental group histology and scaffold fluorescence. Histological sections are stained with methylene blue/basic fuschin. Fluorescent sections illustrate incorporated tetracycline and calcein from injection series.

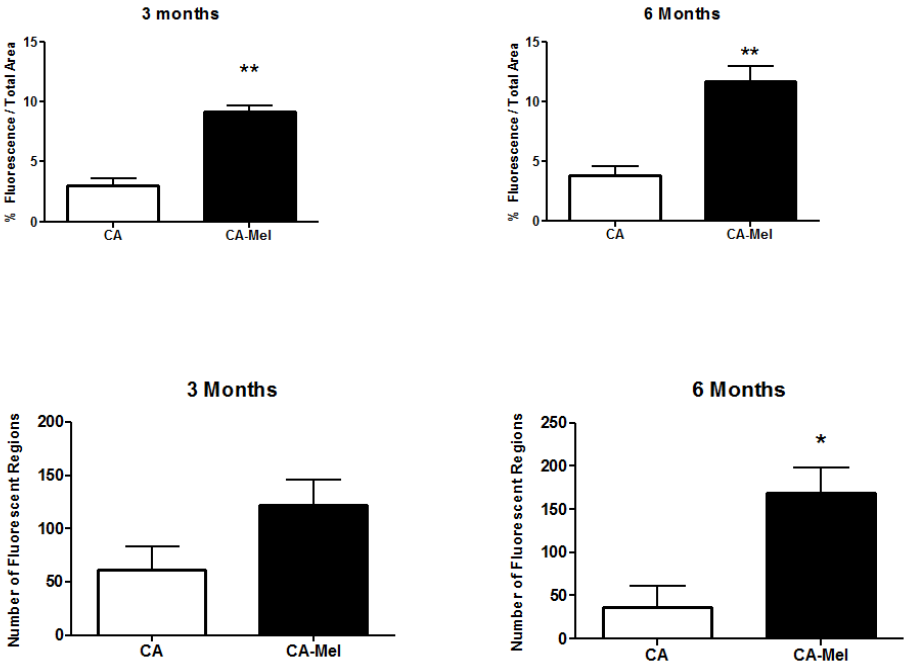

Figure 3. Quantification of percentage of scaffold fluorescence, fluorescent intensity, and the number of regions of fluorescence at three and six months. Groups were compared via 2-way ANOVA with Bonferroni post-hoc t-test

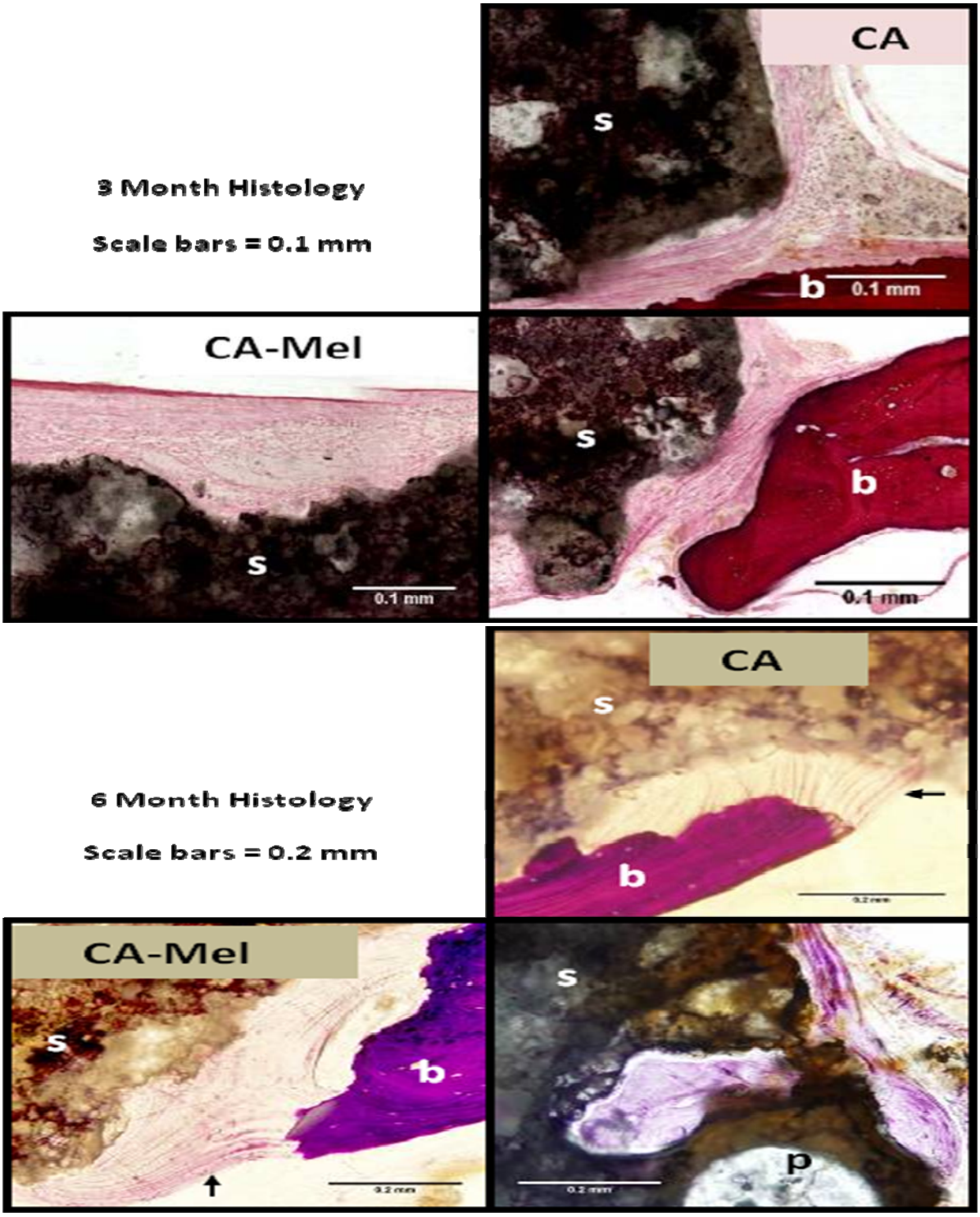

Figure 4. Three and six month photomicrographs of defect bone and scaffold interface showing tissue integration on scaffold surface, tissue infiltration into scaffold pores, and lamellar attachments from bone edge. Histological sections are stained with methylene blue/basic fuchsin. S = Scaffold, b = bone at defect edge, p = scaffold pore, arrow = lamellar attachments.