Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Pittsburgh Tissue Engineering Initiative, Inc.

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 - 12/31/2011

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Charlotte Emig

4. **Grant Contact Person's Telephone Number:** 412-624-5518

5. **Grant SAP Number:** 4100054867

6. **Project Number and Title of Research Project:** 01 - *Acrylic-Bisphosphonate Polymer Mediated Cell Binding to Collagen-Hydroxyapatite Scaffolds*

7. **Start and End Date of Research Project:** 1/1/2011 - 12/31/2011

8. **Name of Principal Investigator for the Research Project:** Richard R. Koepsel PhD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ $9,815

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Koepsel	Principle Investigator	10%	$9,815

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Jose	Post-Doc	5%
D'Souza	Post-Doc	25%
Andersen	Technician	10%
Askarova	Visiting Scientist (PhD)	50%
Adambekov	Visiting student	50%
Yantsen	Visiting student	50%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes____x_____ No_____

If yes, please indicate the source and amount of other funds:
Advanced Regenerative Medicine IV (DoD through PTEI) $13,000

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____X____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Targeted Cell Therapy for Treatment of Osteoporosis	☐NIH ☐ Other federal (specify:_____ _____) X Nonfederal source (specify: _Government of Kazahkstan___)	12-11	$ Not available	$ Not available
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____x_____ No_____

If yes, please describe your plans: We will be part of the grant submitted in Kazakhstan and will submit a grant using background developed here but on a different topic

12. Future of Research Project. What are the future plans for this research project?

This project will continue in Kazahkastan and will diverge here.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No____x_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes____x_____ No_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:
3 visiting scholars from Kazakhstan were trained to do this project all 3 are from and returned to The Center for Life Sciences, Nazarbayev University, Astana Kazakhstan.:
Sholpan Askarova PhD,
Sholkar Adambekov MS,
Yulia Yantzen BS

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____x____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

We were able to establish a collaboration with colleagues in Kazakhstan.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____X_____ No_____

If yes, please describe the collaborations:
Please see # 14 and 15 above.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____x____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Project Abstract from Original submission:

Acrylic-Bisphosphonate Polymer Mediated Cell Binding to Collagen-Hydroxyapatite Scaffolds - Retention of osteoblasts or progenitor cells is an important characteristic for scaffolds designed to enhance bone healing. We have developed a poly-functional polymer that can be attached to cells and direct their binding to bone or bone substitute materials that contain hydroxyapatite. The polymer has a succinimide functionality that allows covalent attachment to cell surface amine groups and pendant bisphosphonate groups that provide specific attachment to the

hydroxyapatite (HA) component of bone tissue. In this project we propose to synthesize biomimetic scaffolding made up of electrospun collagen fibers containing hydroxyapatite nanoparticles. The scaffold will be tested for its ability to bind and retain cells and for its degradability under simulated in vivo conditions.

Project Overview (from original submission)

Scaffold materials are an important part of regenerative medicine. While numerous materials exist for bone repair, few are routinely seeded with cells to enhance remodeling. This project describes the synthesis and testing of a non-woven fabric material made from the components of bone. A fabric composed of micro fibers of collagen with inclusions of nanoparticulate hydroxyapatite will be produced by electrospinning. The ratios of the components used to electrospun the fabrics will be varied to achieve optimized material characteristics of strength, flexibility, porosity, and stability. The fabrics will be analyzed by scanning electron microscopy to determine the porosity of the material. The stability of the material will be measured to determine the porosity of the material. The stability of the material will be measured by the degradability of the material *in vitro* under simulated physiological conditions. The materials should have a degradation profile compatible with bone remodeling activity.

The scaffold material will be tested for its ability to bind to a unique polymer that contains bisphosphonate side groups which bind tightly to hydroxyapatite. The polymer is designed as a bone targeting reagent and has the ability to bind to cells or proteins and bind them to bone materials. The polymer will be assayed for its ability to interact with the scaffold fabrics, and for its ability to bind cells to the fabric. A variant of the polymer which carries a fluorescein moiety will be used in the binding assays. Binding will be followed by fluorescence spectroscopy and by microscopic analysis. The density of cell attachment will be determined by first reacting the polymer with HL60 cells followed by reaction of the cells with the fabrics and counting the bound cells. Mouse osteoblasts cells will be obtained and will be tested for their ability to grow within the scaffolding and for their ability to convert the scaffolding to bone like structures.

Summary of Research Completed:

This report represents results generated from a one year project to direct the binding of cells to specific materials. In this case the materials were intended to be a new type of composite fabric made for the project that would act as a scaffold for binding of osteogenic cells that could then be used as a bone injury repair material. It should be noted that the entire budget for this project was intended to cover approximately 10% of the PI's effort in overseeing, participating in, and reporting on the research. Further, many aspects of the research that were essential to progress on this specific project were funded by other sources. Because this is a highly collaborative effort including polymer synthesis, scaffold synthesis, cell culture, polymer binding to surfaces, microscopic imaging, data analysis and reporting, there were a number of participants whose efforts were coordinated by the PI to achieve results. The results reported here are, thus, the results of the PI's participation in the study while not all were funded by the project. This is not to diminish the contribution of the Formula Grant funding, as without it there would have been no results. This is, rather, disclosure that the results discussed below were not all directly derived from the Formula Grant funds.

The first phase of the project was the synthesis of non-woven microfiber fabric scaffolds for testing as binding targets for a novel bone targeting polymer currently under development in the laboratory. As with any electrospinning process the concentration of the fiber forming material is critical to the consistency of the final product. The goal for this project was to spin collagen fibers with diameters in the 0.5 to 2.0 micron range. Optimization of the collagen fibers revealed that a starting concentration of 4% collagen resulted in a preparation of fibers in the desired range (Figure 1). Analysis of the resulting electrospun materials was by scanning electron microscopy (SEM).

The next step in the process was the incorporation of hydroxyapatite crystals into the collagen fiber mat. This was achieved by adding various concentrations of nano-crystalline HA into the collagen solution. The goal was to incorporate enough HA into the fibers that the crystals would extend beyond the boundaries collagen fibers without disrupting the integrity of the fibers. This would result in materials that had both the composition and structure of healing bone. It was found that the minimum concentration of HA that fit the criteria was 15% HA by weight in the 4% collagen solution. Figure 2 shows SEM pictures of a fiber mat that was electrospun from a 4% collagen solution that contained 15% HA. The nano-particulate HA particles agglomerated into clumps many of which protrude from the surface of the fibers (Figure 2 inset). While many of the agglomerated HA clumps are larger than the diameter of the collagen fibers, few seem to be disrupting the fibers. Additionally few of the HA clumps appear to be at the ends of fibers suggesting that the clumps are in the middle of the fibers and are integral to the fiber structure.

In order to test the ability of a polymer to bind to the collage-hydroxyapatite scaffolds, a di-block polymer was synthesized in a two-step atom transfer radical polymerization (ATRP) strategy. The first block, designed primarily to give length to the polymer, is composed of *N*,*N*-dimethylacrylamide (DMAA) with a small amount of pendant fluorescein O-methacrylate as a tracer. The second block was synthesized using the first block as a macro-initiator and contained DMAA and *N*-acryloyl-6-aminohexanoic acid *N*'-oxysuccinimide ester providing pendant NHS groups for attachment of tissue associating moieties. An end group NHS was provided for cell surface attachment. The polymer was targeted to hydroxyapatite or bone by reacting the pendant NHS groups with an amino bisphosphonate (alendronate). The bisphosphonate polymer was further modified to carry a terminal NHS group which was used to bind the polymer to HL60 cells. The polymer bound cells were shown to bind to hydroxyapatite (HA) crystals and bone fragments.

In order to test the suitability of the polymer for binding to the scaffolds the kinetics of binding to HA were determined in an equilibrium binding assay. Increasing concentrations of polymer were mixed with HA crystals and allowed to bind for 2 hours at 37 ^{0}C. The HA crystals were washed to remove unbound polymer and the polymer HA complex was dissolved in 0.1M HCl. The relative fluorescence was measured and the values of the test samples were calibrated with known polymer concentrations (Figure 3). Non-linear regression analysis gives a value for the dissociation constant (K_d) of 0.45mM. The K_d of the polymer represents binding of the two alendronate moieties per polymer chain. The K_d of the polymer thus fits well with the K_d of 1mM reported for alendronate binding to rat bone fragments. This is in contrast to the K_d of 0.072mM found for alendronate on human bone and indicates that polymer binding to human bone should be much tighter.

Once the polymer was qualified binding assays to the collagen-HA scaffolds were attempted. These assays proved impossible as the natural background fluorescence of the collagen-HA made detection of the polymer binding untenable. Alternative fluorescent tags have been investigated and new synthesis reactions are in process. As an alternative route to qualifying the polymer for binding to scaffolds, the ability of the polymer to bind to cells and to bind cells to HA crystals and bone was investigated.

The inclusion of fluorescein in the polymer allowed visualization of polymer binding to both HA and to cells. Binding of the polymer to HA is shown in Figure 4. Comparison of the white light and fluorescence images of the same microscopic fields shows that all of the HA crystals are coated with polymer. When the control polymer is mixed with the HA (Fig. 4 a and b) only a small background florescence is detected likely due, in part, to nonspecific adsorption of the polymer on the HA in contrast to the results with the active binding polymer (Fig. 4 c and d).

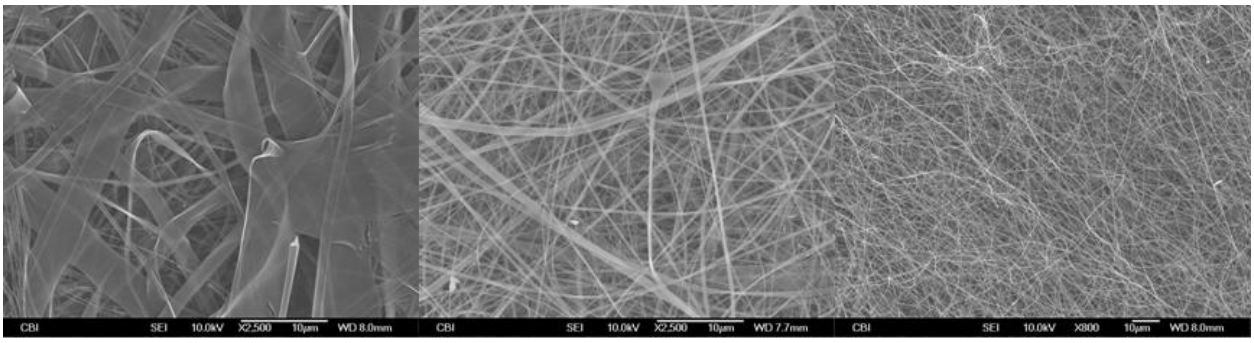
Mixing the active polymer with cells results in the reaction of the polymer NHS group with reactive groups on the cell surface proteins and carbohydrates of the cells. Cells with polymer attached to their surface should then be able to bind to HA and bone through the bisphosphonate groups of the polymer. The HL-60 cell line was used for these experiments because of its low levels of intrinsic adherence to surfaces. Mixing active polymer with HL-60 cells resulted in labeling of all of the cells with polymer (Fig. 5). The optimal concentration of the polymer for adequate binding to the cell surface was found to be 1 mg/ml at an average cell count $2\pm1\text{x}10^5$/ml for 10 minutes at 37 ˚C and pH 8.0.

Modification of cell surface structures can affect the viability of the cells. To test whether polymer binding was cytotoxic, cells were labeled with varying concentrations of polymer and tested for viability and proliferation using the MTT assay (Fig. 6). MTT measures the respiratory activity of cells and is thus a good measure of viability and relative cell number. Comparison of the growth unlabeled cells with the polymer labeled cells showed that the polymer was not significantly cytotoxic.

Active polymer labeled HL-60 cells bind bone fragments (Fig 7). Cells labeled with polymer were mixed with HA crystals and gently washed 3 times by allowing the crystals to settle to the bottom of the tube followed by removal of the supernatant. The mixture of cells and HA crystals was then dropped onto a microscope slide and subjected to gentle pulsatile flow by slowly withdrawing and expelling part of the volume with a micropipette. The cells were stably associated with the HA crystals and not dislodged by the flow conditions.

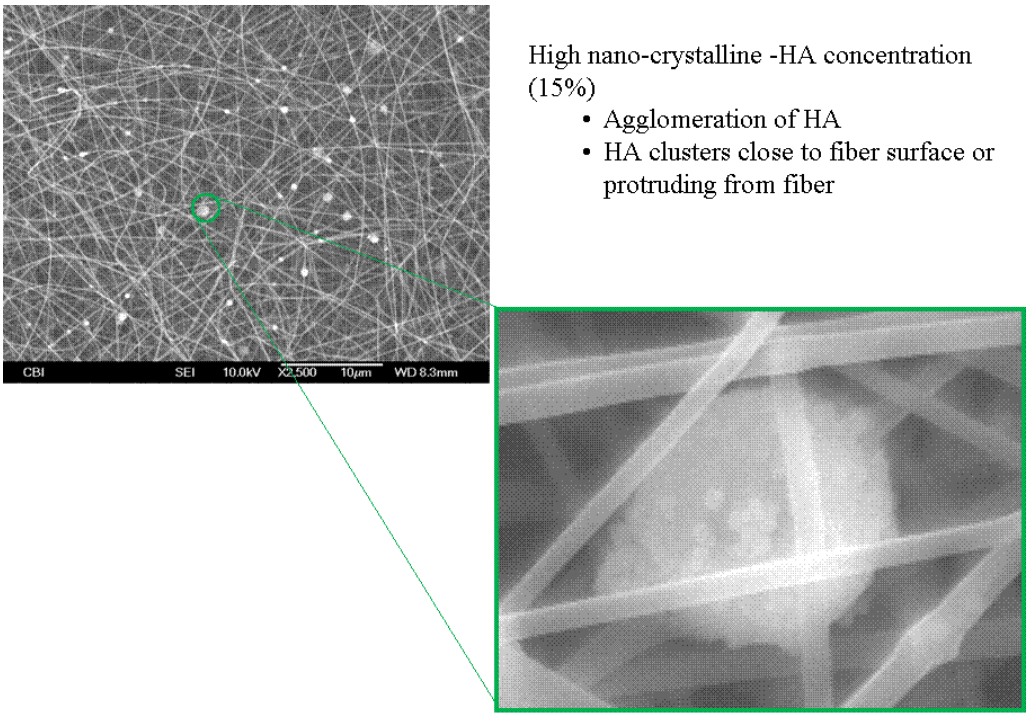
Selective binding of HL-60 cells to bone was shown by comparing cells labeled with control polymer to cells labeled with active polymer (Figure 7). Representative micrographs of the results of labeled cells with bone fragments are shown in Figure 7A and 7B. Additionally, 13 random images from each condition were scanned and the amount of green fluorescence quantified. Comparison of active polymer labeled cells with control (non-binding) polymer (Fig 7C) shows significantly more fluorescence in with the active polymer labeled cells further supporting the visual evidence.

The case for the polymers' ability to bind cells to bone is clear. What remains is to test the cell binding on the scaffolds synthesized for the project and to test the materials *in vivo*.



Collagen -8% Collagen -5% Collagen 4%

Figure 1. Optimization of collagen concentrations by electrospinning



High nano-crystalline -HA concentration (15%)

- Agglomeration of HA
- HA clusters close to fiber surface or protruding from fiber

Figure 2. Electrospun collagen Hydroxyapatite fibers

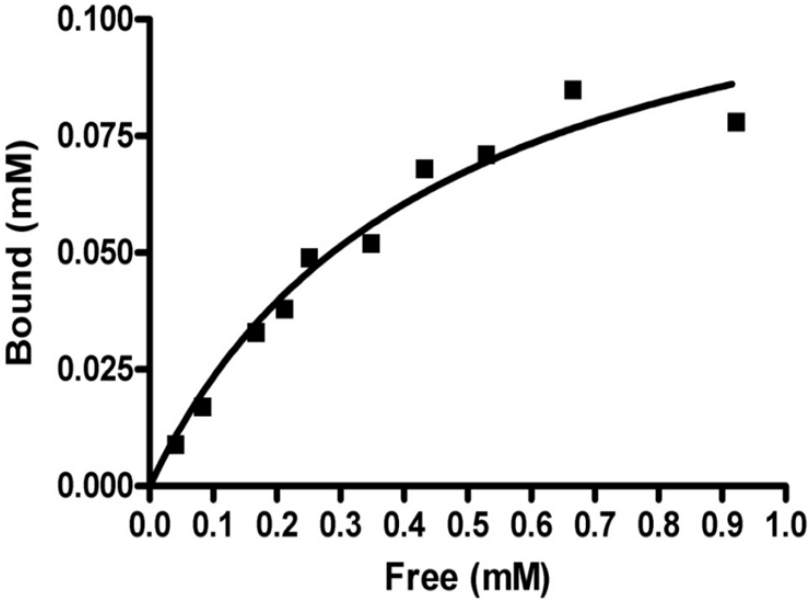


Figure 3

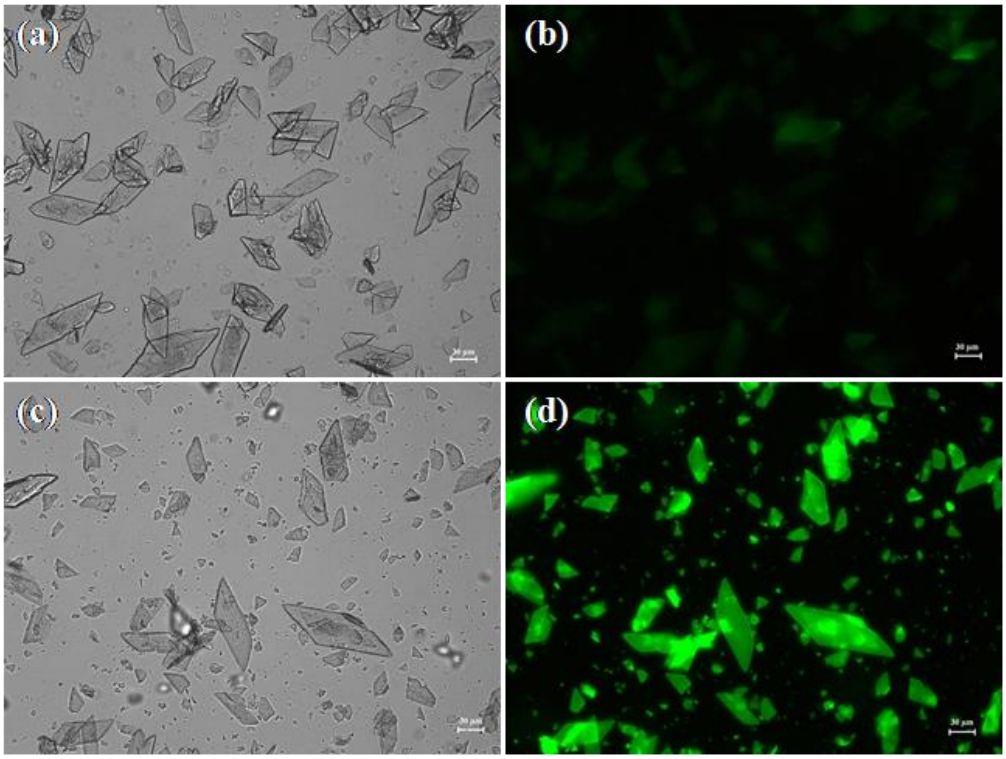


Figure 4

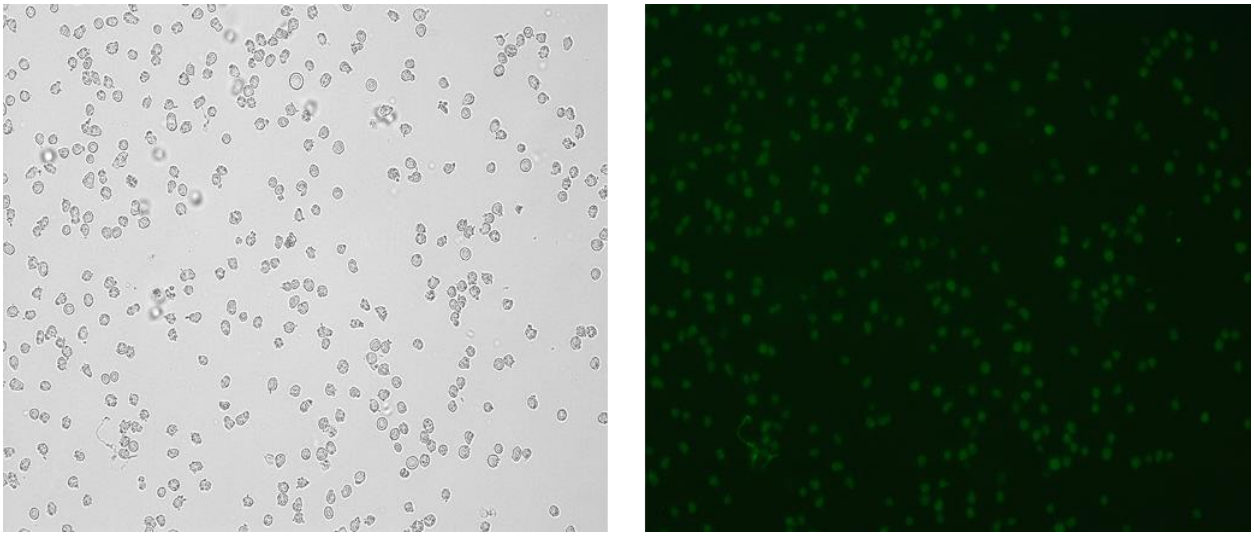


Figure 5

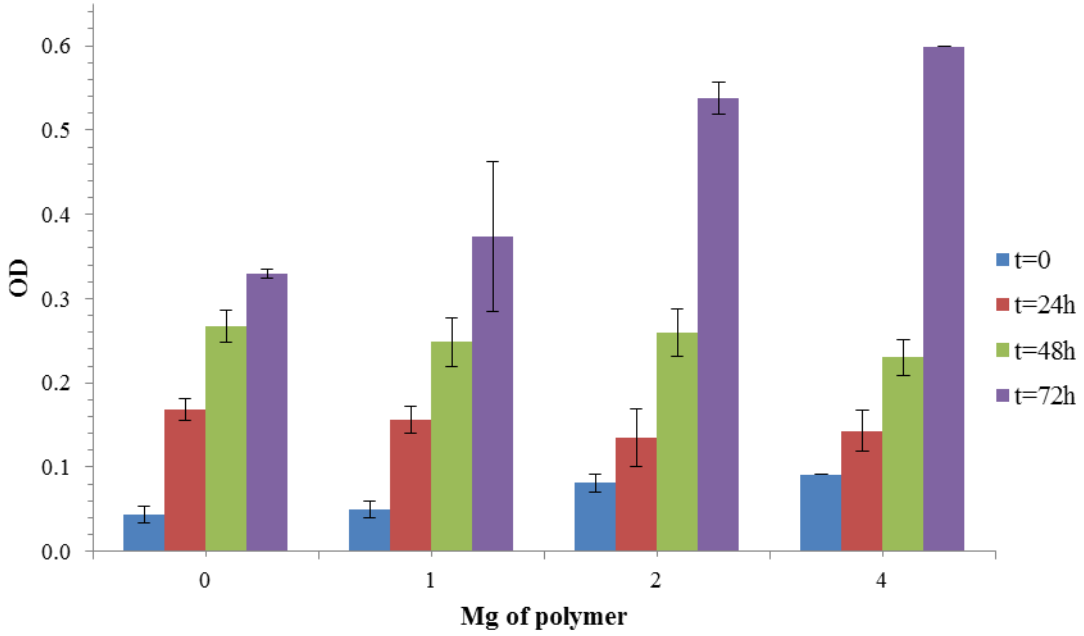


Figure 6

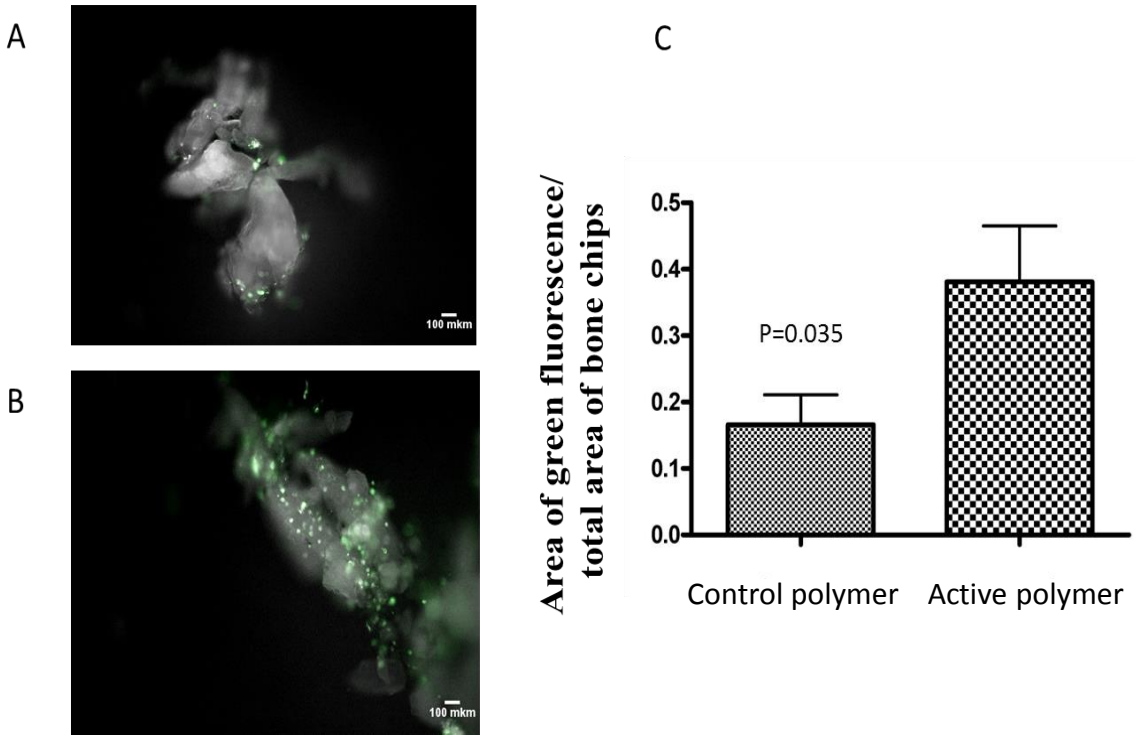

A
B
C
100 mkm
100 mkm
Area of green fluorescence/
total area of bone chips
0.5
0.4
0.3
0.2
0.1
0.0
P=0.035
Control polymer
Active polymer

Figure 7

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
____x__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics

_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___x___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
___x___ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include

the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____x____ No_____

If yes, please describe your plans:

A manuscript is undergoing final editing in advance of submission. We plan to target the journal Biomacromolecules.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis,

or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

We believe that the results to date indicate that the original goals are still achievable. We also believe that the results indicate that in a subset of cases cells could be modified with polymer such that they will partition differentially from the circulation to a target tissue. This could result in a method to direct cells to regenerating tissues, to diseased tissues, or to areas which have become denuded. If the cells were selected for specific properties they could be a means to reinforce the natural regenerative processes.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Polymer directed binding of cells to specific tissues is a novel application that can be further exploited.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
   Yes_____ No____
   If yes, indicate number of patent, title and date issued:
   Patent number:
   Title of patent:
   Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

   If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

   If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____x_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Richard R. Koepsel, Ph.D.	Research Professor
eRA COMMONS USER NAME koepsel	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Wisconsin, Madison WI	BS	1974	Bacteriology
University of Wisconsin, Madison WI	MS	1977	Pharmacognosy
University of Wisconsin, Madison WI	Ph.D.	1981	Bacteriology
University of Pittsburgh, Pittsburgh PA	Post-doc	1982-1987	Molecular Biology

A. Personal Statement

I have over 30 years of experience in research projects involving bacteria and antimicrobials. Over the past 10 years I have worked with Dr Alan Russell studying antimicrobial surfaces and polymeric antimicrobial materials. We have been able to synthesize antimicrobial polyquaternary amines and to characterize their mechanism of action. Additionally we have recently synthesized a new class of antimicrobial materials which generate active halogens using immobilized enzymes acting on glucose and halide salts.

B. Professional Experience

February 1987 – June 1999
Assistant Professor
Department of Microbiology-Biochemistry
School of Dental Medicine
University of Pittsburgh

July 1999 – July 2009
Research Associate Professor
University of Pittsburgh
Department of Chemical and Petroleum Engineering
Center for Biotechnology and Bioengineering
300 Technology Drive
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July 2009 – present
Research Professor
University of Pittsburgh
Department of Surgery
McGowan Institute for Regenerative Medicine
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C. Selected Publications

Jose, M., Marx, S., **Koepsel, R.R.,** Murata, H., Russell, A.J. Direct Electron Transfer in a Mediator-Free Glucose Oxidase-based Carbon Nanotube-Coated Biosensor. Carbon. Submitted.

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Ravikumar T, Murata H, **Koepsel RR**, and AJ Russell. Surface-Active Antifungal Polyquaternary Amine. *Biomacromolecules* 7: 2762-2769, 2006

Lee SB, **Koepsel RR**, Russell AJ Surface dispersion and hardening of self-assembled diacetylene nanotubes *NANO LETTERS* 5: 2202-2206, 2005

Zhu T, Pan Z, Domagalski N, **Koepsel R**, Ataai MM, Domach MM. Engineering of Bacillus subtilis for enhanced total synthesis of folic acid. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* 71: 7122-7129, 2005

Lee SB, **Koepsel RR**, Morley SW, Matyjaszewski K, Sun YJ, Russell AJ. Permanent, nonleaching antibacterial surfaces. 1. Synthesis by atom transfer radical polymerization BIOMACROMOLECULES *5:877-882* 2004

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