Intelligent Polymers for Tissue Engineering Applications

THE UNIVERSITY OF TEXAS AT AUSTIN
Cockrell School Biomedical Engineering

Marissa E. Wechsler[1,2] and Nicholas A. Peppas[1,2,3,4,5]

UT Austin | Portugal
International Collaboratory for Emerging Technologies, CoLab

[1]Department of Biomedical Engineering, [2]Institute for Biomaterials, Drug Delivery, and Regenerative Medicine, [3]Department of Chemical Engineering, [4]Department of Surgery, Dell Medical School, [5]College of Pharmacy, The University of Texas at Austin, Austin, TX, USA

INTRODUCTION

Approximately half a million bone defects require tissue graft reconstruction in the Unites States each year. The gold standard for treatment is the use of autologous bone grafts, however, these present several limitations with the most predominant being availability and donor-site morbidity. In order to overcome these limitations, there is a need to develop novel alternatives or adjuncts to traditional methods used to promote new bone tissue formation.

Bone tissue engineering aims to induce tissue regeneration by means of combining biomaterials, cells, and various stimulatory signals. Use of biological signals and cells to promote the native healing response of the host may address many disadvantages of current approaches for bone tissue repair. Localized delivery of osteoinductive factors (e.g., growth factors, peptides, and small molecules) remains a major challenge in bone tissue engineering to promote regeneration and repair of damaged tissues. Delivery of select bone morphogenetic proteins (BMPs) has shown great therapeutic potential (specifically, BMP-2 and BMP-7), however, current delivery methods require supraphysiological concentrations to obtain desired effects and experience inadequate retention at the site of delivery. In contrast, the use of small molecules to induce bone regeneration has not been fully explored and displays several advantages over the use of growth factors (including small size, high stability and non-immunogenicity). Local administration of osteoinductive small molecules can be achieved through incorporation within osteoconductive materials, such as, bone tissue engineered scaffolds. However, optimal carriers for small molecule delivery within these scaffolds remain partially unknown.

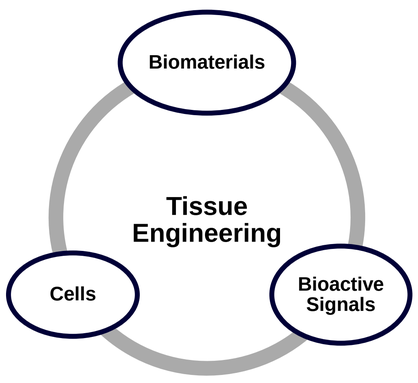

Figure 1. Tissue engineering triad

Growth Factors	Peptides	Small Molecules
• Advantages • Specific • Stimulate cell proliferation and differentiation following naturally occurring mechanisms	• Advantages • Small size • Low immunogenicity • Self-assembling possibilities • Ease of production • Stable	• Advantages • Physico-chemically well defined • Minimized costs and risk of contamination • Non-immunogenic • Stable
• Disadvantages • Unstable • Impurities • Supraphysiological concentrations required • High cost	• Disadvantages • Unstable • High cost • May provoke immune response	• Disadvantages • May penetrate non-target cells • Non-specific adverse effects

Figure 2. Advantages and disadvantages of growth factors, peptides and small molecules
***Note*: Figure adapted from Balmayor, Advanced Drug Delivery Reviews (2015).**

GOAL

To develop an intelligent polymer based platform for the delivery small molecules to promote functions of bone cells pertinent to new bone tissue formation.

SPECIFIC AIMS

Aim 1. Synthesize and characterize polymeric systems for small molecule delivery

Particles will be synthesized via solution polymerization. N-isopropylacrylamide (NIPAM) will be co-polymerized with methacrylic acid (MAA), and/or benzyl methacrylate (BZMA), or 2-hydroxyethyl methacrylate (HEMA) using N,N'-methylenebisacrylamide as the crosslinker.

Characterization techniques:
- Nuclear magnetic resonance spectroscopy
- Fourier transform (FT-IR) infrared spectroscopy
- Dynamic light scattering (DLS)
- Transmission electron microscopy (TEM)
- Rheology

Aim 2. Analyze the load and release of small molecule loaded polymer systems

Purmorphamine

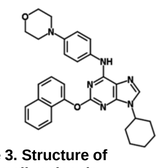

Figure 3. Structure of the small molecule purmorphamine

Phenamil

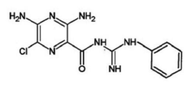

Figure 4. Structure of the small molecule phenamil

Loading methods:
- *In situ* loading
- Post formulation loading
- Molecular imprinting

Realease:
- Sustained or burst release at physiological conditions
  - Diffusion-controlled
  - Swelling-controlled
  - Chemically-controlled

Aim 3. Assess the performance of developed small molecule carriers using *in vitro* models

Cell types to be examined:
- Bone-marrow derived murine mesenchymal stem cells
- Murine osteoblasts
- Human osteoblasts
- Bone-marrow derived human mesenchymal stem cells

In vitro assays to be performed:
- Cytotoxicity/viability
- Cell proliferation
- Cell differentiation
  - Gene expression analysis
  - Protein deposition
- Mineralization

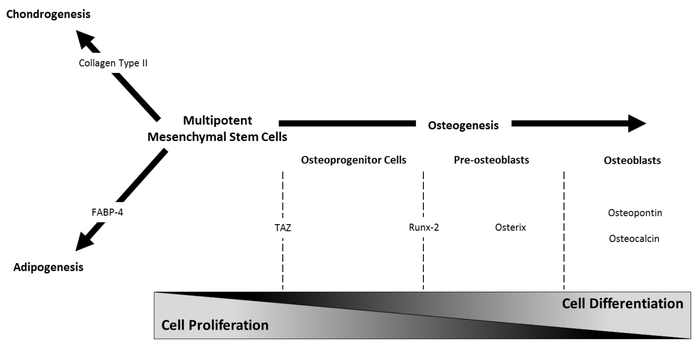

Figure 5. Osteodifferentiation pathway of mesenchymal stem cells
***Note*: Figure adopted from Wechsler *et al*., Tissue Eng Part C Methods (2015).**

Aim 4. Evaluate the *in vivo* capability of the loaded small molecule polymeric systems

A bone defect model will be used to evaluate the *in vivo* performance of the small molecule loaded polymeric systems. Species to be examined include either mouse, rat or rabbit.

PRELIMINARY RESULTS

Nanoparticle characterization (FT-IR/TEM/DLS)

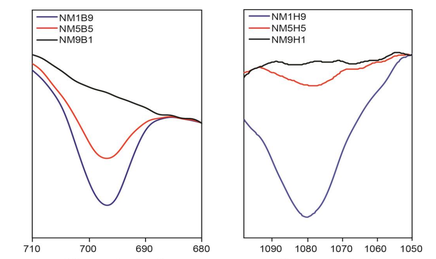

Figure 6. Representative FT-IR spectroscopy of P(NIPAM-co-MAA-co-BZMA or HEMA) nanoparticles at various concentrations
***Note*: Figure courtesy of Heidi R. Culver.**

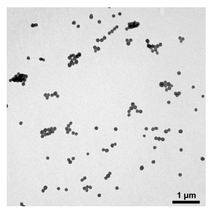

Figure 7. Representative TEM image of P(NIPAM-co-MAA) nanoparticles
***Note*: Nanoparticles were stained with 2% uranyl acetate. Figure courtesy of Heidi R. Culver.**

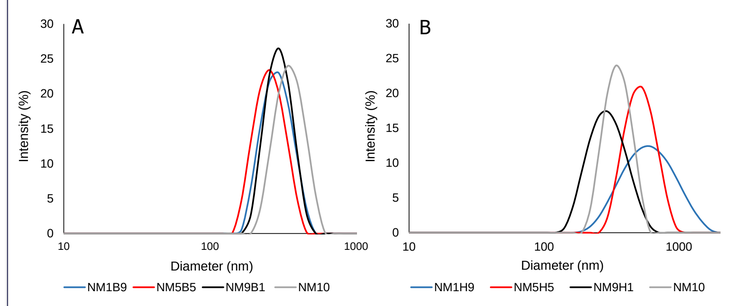

Figure 8. Representative intensity-weighted particle size distribution of synthesized nanoparticles
A: DLS of P(NIPAM-co-MAA) and P(NIPAM-co-MAA-co-BZMA) at various concentrations; B: DLS of P(NIPAM-co-MAA) and P(NIPAM-co-MAA-co-HEMA) at various concentrations

SUMMARY

Specific aims are currently a work in progress. Various polymeric nanoparticles have been synthesized including the following: P(NIPAN-co-MAA), P(NIPAM-co-MAA-co-BZMA) and P(NIPAM-co-MAA-co-HEMA). Synthesis of nanogels were confirmed by Fourier transform infrared spectroscopy. Nanoparticle size was determined using dynamic light scattering. Results show successful synthesis of the desired polymer formulations. Synthesized nanogels can thus be used to investigate the load and release of small osteoinductive molecules, followed by assessing the *in vitro* and *in vivo* performance of the loaded polymeric systems. Successful delivery of these molecules within tissue engineered constructs could have major impact in bioengineering and in specific therapeutic modalities in the clinical milieu.

ACKNOWLEDGEMENTS

The authors would like to thank Ms. Heidi R. Culver and Dr. Julia Vela-Ramirez for assistance with various aspects of this project, as well as, the National Science Foundation Graduate Research Fellowship which provided support to MEW and the UT Portugal CoLab Program.

REFERENCES

- Balmayor ER. Targeted delivery as key for the success of small osteoinductive molecules. Adv Drug Deliv Rev. 2015;94:13-27.
- Wechsler ME, Hermann BP, Bizios R. Adult human mesenchymal stem cell differentiation at the cell population and single-cell levels under alternating electric current. Tissue Eng Part C Methods. 2015.
- Vo TN, Kasper FK, Mikos AG. Strategies for controlled delivery of growth factors and cells for bone regeneration. Adv Drug Deliv Rev. 2012; 64(12): 1292–1309.
- Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng. 2012; 40(5): 363–408.

IBDR | INSTITUTE FOR BIOMATERIALS, DRUG DELIVERY & REGENERATIVE MEDICINE