

## INTRODUCTION

# ENGINEERING THE TISSUE EXTRACELLULAR MATRIX WITH HYBRID BIOMATERIALS

Esmail Jabbari\*

*Biomimetic Materials and Tissue Engineering Laboratory, Department of Chemical Engineering, University of South Carolina, Columbia, SC 29208, USA*

*\*E-mail: jabbari@engr.sc.edu*

### 1. Introduction

Synthetic materials are finding widespread applications as a replacement for tissue extracellular matrix in medicine.<sup>1</sup> These include, among others, biomaterials for total hip and knee replacement, bone grafts, intraocular lenses, vascular grafts, breast implants, sutures, artificial heart, vascular stents, dental implants, as a scaffold in cranial and maxillofacial reconstruction, and as a carrier in drug delivery. Autologous grafts, the gold standard for tissue replacement, have limited supply and suffer from donor site morbidity. Allografts are limited by reduced rates of graft incorporation. The flexibility in the design of synthetic materials allows the synthesis of a wide range of tissue replacement grafts with varying physical, mechanical, biologic and degradation properties to suit various medical applications. However, there are also limitations, especially in regenerative medicine, in the use of synthetic materials. For example, titanium cages coated with collagen and loaded with osteoinductive factors are being used in spine fusion but the metallic cage shield stress from the regenerating region during the remodeling phase resulting in lower than expected bone density.<sup>2</sup> Bioactive ceramics augmented with bioactive factors promote mineralization and bone formation but their drawbacks are fatigue fracture and low tensile strength.<sup>3</sup> Synthetic hydrolytically degradable polymers like poly(L-lactide), poly(glycolide), and their lactide-co-glycolide copolymers can provide some structural support to the regenerating region and their surface can be treated to support cell adhesion, but these hydrophobic matrices have limited ability to support solubilization and diffusion of proteins and their interaction with the cellular environment. Hydrogels like poly(ethylene glycols) (PEG), due to their

hydrophilicity and high water content, exhibit excellent biocompatibility, and immobilized biomolecules retain higher biological activity within hydrogels<sup>4</sup> but their low strength and soft tissue compression limits their application in regenerative medicine.

It is now well-established that synthetic materials alone or in combination with soluble growth factors can not provide the microenvironment and the signaling factors required to initiate the cascade of cell migration, homing, adhesion, differentiation, matrix formation and remodeling in tissue regeneration. Furthermore, synthetic macromolecules do not possess in their structure the pattern of weak non-polar, polar, hydrogen bonding, and electrostatic interactions to produce functional specificity for drug targeting, imaging, or biological sensing. On the other hand, scaffolds produced from bioactive sequences alone are potentially immunogenic and have low metabolic stability in-vivo.<sup>5</sup> An attractive alternative is to use synthetic materials grafted/conjugated/hybridized with bioactive motifs, originating from the amino acid and carbohydrate sequences on proteoglycans and glycoproteins in the ECM. These biomimetic materials and structures possess biological recognition motifs as well as desired engineering properties. In addition, synthetic materials hybridized with bioactive motifs represent a multivalent form of the ligand for interaction with cluster of biological receptors, resulting in stronger interaction or stimulating particular subsets of receptors.<sup>6</sup> In the following sections, an overview of the synthesis, characterization, structure, and applications of hybrid biomaterials is presented, followed by the book structure.

## 2. Synthesis

Poly(lactide-co-glycolide) (PLGA) is the most widely used biodegradable polymer in the biomedical field. It is used in fabrication of pre-formed tissue engineering scaffolds in regenerative medicine and nano-/micro- particles in drug delivery because its degradation products, lactic and glycolic acids, are resorbed through the metabolic pathways. Furthermore, the flexibility in its design allows the synthesis of a wide range of polymers with varying mechanical, biologic and degradation properties to suit various applications. To further expand the application of PLGA in minimally-invasive procedures, a star lactide-co-glycolide (SLGA) macromer consisting of a hydrophilic multi-arm (2, 3, 4, 6, or 8) polyethylene glycol (PEG) core with very short lactide-glycolide (LG) chains terminated with an acrylate group attached to each arm is designed, as shown in Figure 1. The ethylene oxide core provides hydrophilicity and controlled water uptake to improve biomolecule and cell viability. The short LG chains provide

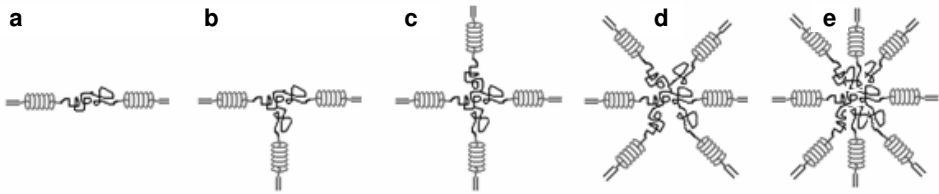


Fig. 1. The molecular structure of the star lactide-co-glycolide acrylate (SLGA) macromer with a polyethylene glycol (PEG) core. The macromer consists of a multi-arm PEG core (blue) with a lactide-co-glycolide (LG) segment (green) terminated with an acrylate group (red) at one end attached to each arm of the PEG core. The distinguishing feature of this macromer is the very short LG segments allowing the macromer to crosslink, via the unsaturated acrylate groups, to form a solid 3D network. The rate of network formation and crosslink density depend on the number of arms and the macromer molecular weight, respectively. The modulus depends on the number of arms and PEG:LG ratio. The water uptake of the crosslinked network depends on the ratio of hydrophilic PEG core to hydrophobic LG segments and the extent of crosslinking. The rate of degradation of the crosslinked network depends on the ratio of lactide to glycolide in LG segments. (a), (b), (c), (d), and (e) show the structure of the SLGA macromer with 2-arm (linear), 3, 4, 6, and 8 arms, respectively.

degradability and hydrophobicity to control water uptake while the unsaturated acrylate groups provide functionality for crosslinking. The rate of network formation is controlled by the number of arms on each macromer. The crosslink density can be controlled by the number of arms and the macromer molecular weight. The water uptake is controlled by PEG:LG ratio and crosslink density. The modulus of the crosslinked SLGA depends on PEG:LG ratio, number of arms, and macromer molecular weight. The degradation characteristic depends on lactide to glycolide ratio in the LG units and the PEG:LG ratio. Some SLGA compositions spontaneously self-assemble to form hydrolytically degradable nanoparticles for drug delivery. The unsaturated acrylate groups or the hydroxyl end groups of SLGA can be utilized for hybridization with bioactive motifs.

Different reaction schemes for conjugation of synthetic macromers to bioactive motifs are shown in Figure 2. Macromers with hydroxyl end-groups can be succinimide-functionalized by treating with *N,N'*-Disuccinimidyl carbonate (DSC) and conjugated with lysine-terminated peptides by the reaction of succinimide end-group of the macromer with amine group of the lysine, as shown in reaction scheme (a) in Figure 2.<sup>7</sup> Carboxylic-acid terminated macromers can be conjugated by the reaction between amine group of the peptide with carboxylic acid end-group of the macromer in the presence of *N,N'*-diisopropylcarbodiimide (DIC) coupling agent and *N,N*-dimethylaminopyridine (DMAP) catalyst, as shown in Figure 2(b). Michael's addition reaction can be

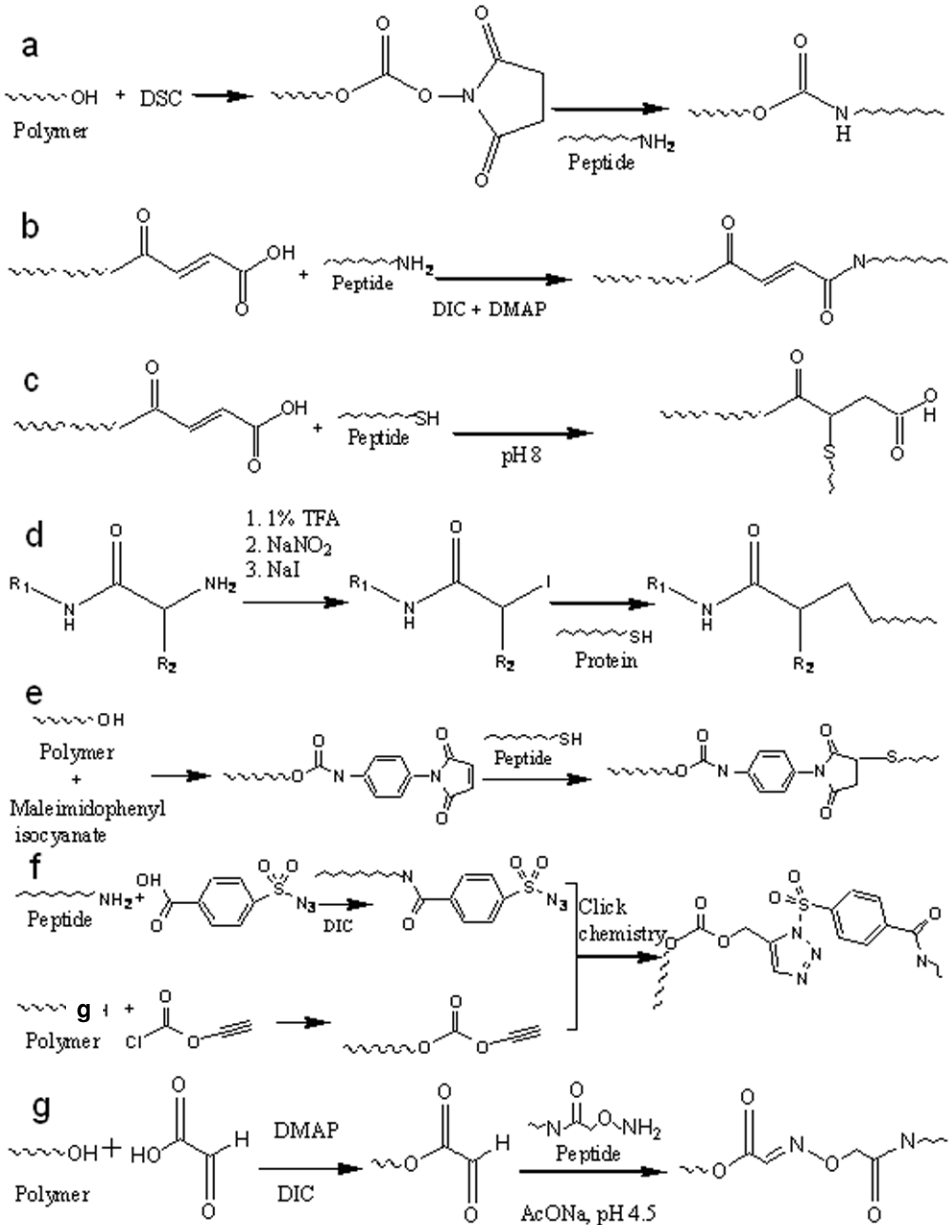


Fig. 2. Reaction schemes for conjugation: (a) succinimide-terminated macromer with lysine-terminated peptide; (b) carboxylic acid-terminated macromer with amine group of peptide; (c) unsaturated group-terminated macromer with sulfhydryl group of cysteine-functionalized peptide; (d) iodine-functionalized macromer with thiol-containing peptide; (e) maleimide-functionalized macromer with thiol-containing peptide; and (f) propargyl-functionalized macromer with azide-functionalized peptide; (g) aldehyde-functionalized macromer with amine group of peptide.

used to conjugate cysteine-functionalized peptides to acrylate-terminated macromers, as shown in Figure 2(c).<sup>8</sup> Another method is selective iodination of the peptide at the N-terminal using nitrite catalysts, followed by the reaction of iodinated peptide with thiol-containing compounds such as N-acetylcysteine and glutathione to form peptide conjugates, as shown in Figure 2(d).<sup>9</sup> Bioactive motifs with free thiol functionality can be conjugated to macromers by the thiol-maleimide conjugation. In this approach, p-maleimidophenyl isocyanate is used for functionalization of hydroxyl-terminated macromer, as shown in reaction (e) of Figure 2.<sup>10</sup> The “click reaction” between the azide group on the peptide and propargyl group on the macromer can also be used for conjugation, as shown in reaction (f) of Figure 2.<sup>11</sup> In “click reaction” the peptide is functionalized with an azide group by reacting with 4-carboxybenzenesulfonazide while the macromer is functionalized with a propargyl group by reacting with propargyl ester chloride. The “Oxime” reaction between an aldehyde-functionalized macromer with the amine group of the peptide can be used for conjugation, as shown in Figure 2(g).<sup>12</sup>

### 3. Characterization

The chemical structure of the macromer can be characterized by nuclear magnetic resonance (NMR) while the molecular weight distribution can be characterized by gel permeation chromatography (GPC) and mass spectrometry (MS). NMR is particularly useful for determining the ratio of different monomers in the macromer. The sequence distribution of the hybrid macromer can be determined by matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry. Size exclusion chromatography combined with MALDI mass spectrometry (SEC/MALDI) or pulsed gradient spin-echo (PGSE) NMR combined with MALDI can be used to accurately determine molecular weight distribution of the macromers.<sup>13</sup> The secondary structure of the hybrids can be characterized by circular dichroism (CD; 200-300 nm range) and Fourier Transform Infrared Spectrometry (FTIR; 1500-1800 nm range). The interaction of the macromer with conjugated peptide can be studied by x-ray reflectivity as well as CD and FTIR. Site-specific iodine labeling can be used to determine topology of the peptide within the conjugate. The effect of amino acid sequence on morphology of the peptide conjugate can be imaged with scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) at micro-, nano-, and angstrom-scale resolution, respectively. In-vitro bioactivity of the hybrids can be directly measured by two-photon excitation fluorescence cross-correlation spectroscopy (TPE-FCCS)

coupled with fluorescence correlation spectroscopy (FCS).<sup>14</sup> The Kaiser test can be used to quantify the density of the peptides conjugated to a substrate.<sup>15</sup> In-vitro toxicity of the hybrids can be determined by cell count after exposure, and their macrophage cell uptake can be quantified by live cell confocal microscopy and flow cytometry. Radioisotope labeling can be used for quantitative measurement of in-vivo biodistribution with a gamma scintillation counter.

#### 4. Structure

Bioactive motifs conjugated to synthetic macromers can alter the nano-scale structure of these biomaterials. The side groups of the amino acids in the peptide can dictate secondary structures and preference for a specific shape in the aggregates. For example, the synthetic macromer poly(lactide fumarate) (PLAF), due to its hydrophobic nature forms solid nanospheres with a relatively wide size distribution range (50-800 nm) in combination with the amphiphilic poly(lactide-ethylene oxide fumarate) (PLEOF) macromer, as shown in Figure 3.<sup>16</sup> The amino acid sequence Val-Val-Val-Val-Val-Val-Lys-Lys (V6K2) is known to self-assemble into vesicles (hollow spheres) with very narrow size distribution (see Figure 3).<sup>17</sup> Interestingly, when the V6K2 is conjugated to PLAF macromer via Michael's addition reaction, the hybrid macromer self-assembles into hollow spheres with a very narrow size distribution, even in the absence of the amphiphilic PLEOF macromer, as shown in Figure 3.<sup>18</sup> Cryogenic scanning electron microscopy images of the V6K2-PLAF hybrids demonstrate that the vesicles have a structure similar to that of V6K2 peptide. These results clearly

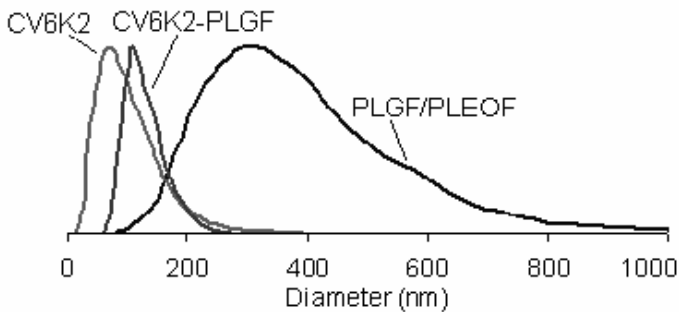


Fig. 3. Particle size distribution of V6K2-PLGF nanoparticles (red) compared to V6K2 vesicles (green) and PLGF stabilized with the amphiphilic poly(lactide-co-ethylene oxide fumarate) (PLEOF) macromer.

demonstrate that short peptide chains can dominate the nanostructure of the hybrids, producing novel biomaterials possessing unique nanostructures as well as biological recognition motifs.

## 5. Applications

Synthetic biomaterials conjugated with bioactive motifs have exciting applications in molecularly targeted diagnosis and imaging, targeted delivery of chemotherapeutic agents to tumor microenvironment, targeted antigen delivery to the most efficient dendritic cells to initiate an antigen-specific immune response in vaccination, receptor-mediated intracellular delivery using cell

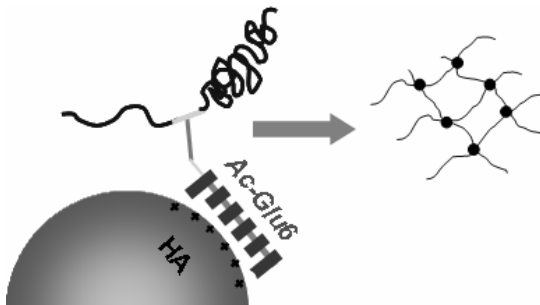


Fig. 4. Schematic diagram showing ionic and covalent interaction of an acrylate-functionalized glutamic acid sequence (red), a sequence in the terminal region of the osteonectin glycoprotein, with apatite (HA) crystals (gray) and the polymer matrix (blue), respectively, to produce a peptide-reinforced biocomposite. The reinforcement effect of the Glu6 peptide is modulated by the size of the HA crystals and it is specific to the glutamic acid sequence.

penetrating peptides in gene and siRNA delivery, controlling cellular expression in regenerative medicine, and in the design of mechanically robust biocomposites. Bioactive motifs based on amino acid sequences of the ECM proteins can be utilized to design peptide-reinforced composites in skeletal tissue engineering. For example, a glutamic acid sequence from the osteonectin glycoprotein is believed to be involved in linking the collagen network to the apatite (HA) crystal phase in the bone matrix.<sup>19</sup> When a glutamic acid sequence with ionic affinity to HA crystals, as shown in Figure 4, is functionalized with an acrylate group (Ac-Glu6) to covalently link the peptide to a poly(lactide-ethylene oxide-fumarate) (PLEOF) polymer matrix, it strongly affected the modulus of the composite matrix.<sup>20</sup> Addition of Ac-Glu6 peptide to nano-HA crystals increased modulus by ten-fold and the reinforcement effect was specific to the glutamic

acid sequence (a glycine or lysine sequence did not have a similar effect). These findings demonstrate that hybrid biomaterials are potentially attractive for developing robust biomimetic composites for skeletal tissue regeneration.

In regenerative medicine, the synthetic scaffold is required to initiate and guide the cascade of cell differentiation and matrix formation. For example, reconstruction of large skeletal defects is limited by the lack of vascularization in the interior parts of the implanted biomaterial. Although the addition of soluble growth factors like bone morphogenetic proteins (BMPs) can substantially improve the extent of mineralization, their loss by diffusion away from the

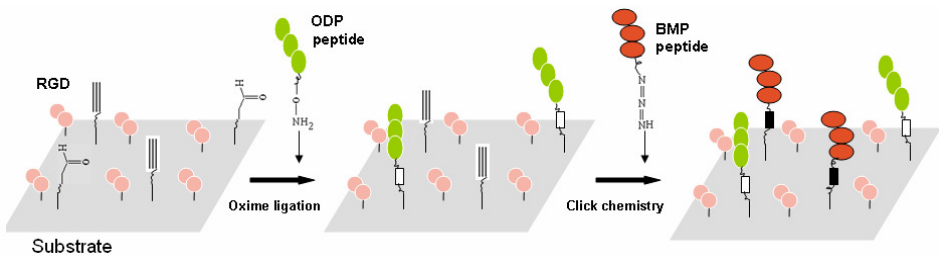


Fig. 5. Schematic diagram showing ligation of a substrate with multiple bioactive peptides by acrylation (RGD peptide), oxime reaction (osteopontin-derived peptide; ODP), and click reaction (peptide based on bone morphogenetic protein; BMP peptide).

intended site of regeneration, degradation, and soft tissue compression requires high doses to induce bone formation. Such high doses cause side effects such as bone overgrowth, abnormal vascular growth, and immunological reactions. An attractive alternative is to graft peptides, based on the active domains of osteogenic and vasculogenic growth factors, to a synthetic biomaterial substrate to initiate the process of vascularized osteogenesis. As an example, when the integrin binding focal-adhesion RGD peptide and a bioactive peptide from the recombinant human bone morphogenetic protein-2 (BMP peptide) were grafted to a synthetic biodegradable substrate, the extent of mineralization (measured by calcium content) of seeded mesenchymal stem cells increased by 12-fold compared to the inert substrate, as shown in Figure 5.<sup>15</sup> Furthermore, when a vasculogenic peptide derived from osteopontin protein was grafted to the substrate, parallel sheets of cells positive for PECAM was observed. These results demonstrate that synthetic hybrid biomaterials can mimic the cell-matrix interactions required for differentiation and maturation of stem cells into multiple lineages.

Another exciting application of hybrid biomaterials is in targeted tumor delivery. Intravenous administration of antitumor drugs allows targeted delivery to tumor vasculature but these less invasive systemic methods are limited by non-selectivity of antitumor drugs to normal cells. It is well established that chemotherapeutic drugs loaded into nanoparticles with narrow size distribution and diameter <200 nm, and conjugated with ligands that bind with high specificity to tumor vasculature can significantly reduce the undesired side effects of chemotherapy. In this regard, hybridization with bioactive motifs can confer biological recognition motifs as well as engineering properties to self-assembled synthetic nanoparticles for targeting to tumor microenvironment. For example, NPs self-assembled from a blend of poly(lactide fumarate) and poly(lactide-co-ethylene oxide fumarate) (PLAF/PLEOF) possess a wide 50-800 nm particle size distribution and have relatively low efficiency of crossing the leaky tumor vasculature. But hybridization of the PLAF macromer with V6K2 peptide produces NPs with very narrow size distribution (50-150 nm), as shown in Figure 3, that can potentially cross the tumor vasculature with high efficiency while delivering the chemotherapeutic agent at a sustained rate during the course of chemotherapy.<sup>18</sup> Interestingly, the hybridized V6K2-PLAF NPs, due to specific electrostatic interactions, show >90% uptake after 2 h by tumor cells.

These and many other examples in this book show that synthetic macromers hybridized with bioactive motifs are very promising as the next generation of biomaterials for biological recognition, imaging, sensing, targeting, and guiding the process of tissue regeneration.

## **6. Book Structure**

This book is divided into three sections. In the first section, synthesis and characterization, conformation, self-assembly, micro-/nano- structure, and host response of hybrid biomaterials are covered. The second section is devoted to the applications of hybrid biomaterials in drug and intracellular delivery and vaccination. The third section is devoted to tissue engineering applications of hybrid biomaterials including cell adhesion, control of the stem cell niche, cartilage regeneration, neural and vascular tissue engineering, and dynamic cell culture systems for functionalized biomaterials. Undoubtedly, biologically-responsive hybrid biomaterials play a vital role in the design of biologics and medical devices. The salient feature of this book that sets it apart from other published books on Biomaterials is that synthesis, characterization, structure-activity, 3D assembly/fabrication, and applications of hybrid biomaterials are covered in one volume.

## References

1. Peppas NA, Langer R, New challenges in biomaterials. *Science* 263(5154):1715-1720 (1994).
2. Ohyama T, Kubo Y, Iwata H, Taki W, Beta-tricalcium phosphate combined with recombinant human bone morphogenetic protein-2: A substitute for autograft, used for packing interbody fusion cages in canine lumbar spine. *Neurol. Med. Chir.* 44:234-241 (2004).
3. Akamaru T, Suh D, Boden SD, Kim HS, Minamide A, Louis-Ugbo J, Simple carrier matrix modifications can enhance delivery of recombinant human bone morphogenetic protein-2 for posterolateral spine fusion. *Spine* 28(5):429-34 (2003).
4. Slaughter BV, Khurshid SS, Fisher OZ, Ali Khademhosseini A, Peppas NA, Hydrogels in regenerative medicine. *Adv. Mater.* 21:1-23 (2009).
5. Latham PW, Therapeutic peptides revisited. *Nat. Biotechnol.* 17(8):755-757 (1999).
6. Rossi EA, Sharkey RM, McBride W, Karacay H, Zeng L, Hansen HJ, Goldenberg DM, Chang CH, Development of new multivalent-bispecific agents for pretargeting tumor localization and therapy. *Clin. Cancer Res.* 9(10):3886S-3896S (2003).
7. Fernandez-Megia, E, Novoa-Carballal R, Quinoa E, Riguera R, Conjugation of bioactive ligands to PEG-grafted chitosan at the distal end of PEG. *Biomacromolecules* 8(3):833-842 (2007).
8. Lutolf MP, Tirelli N, Cerritelli S, Cavalli L, Hubbell JA, Systematic modulation of michael-type reactivity of thiols through the Use of charged amino acids. *Bioconj. Chem.* 12(6):1051-1056 (2001).
9. Deng HT, Nitrite-assisted peptide iodination and conjugation. *J. Pept. Sci.* 13(2):107-112 (2007).
10. Ananda K, Nacharaju P, Smith PK, Acharya SA, Manjula BN, Analysis of functionalization of methoxy-PEG as maleimide-PEG. *Anal. Biochem.* 374(2):231-242 (2008).
11. Ossipov DA, Hilborn J, Poly(vinyl alcohol)-based hydrogels formed by "Click Chemistry". *Macromolecules* 39(5):1709-1718 (2006).
12. Dirksen A, Dawson PE, Rapid oxime and hydrazone ligations with aromatic aldehydes for biomolecular labeling. *Bioconj. Chem.* 19(12):2543-2548 (2008).
13. Mazarin M, Viel S, Allard-Breton B, Thevand A, Charles L, Use of pulsed gradient spin-echo NMR as a tool in MALDI method development for polymer molecular weight determination. *Anal. Chem.* 78(8):2758-2764 (2006).
14. Swift JL, Burger MC, Massotte D, Dahms TES, Cramb DT, Two-photon excitation fluorescence cross-correlation assay for ligand-receptor binding: Cell membrane nanopatches containing the human mu-opioid receptor. *Anal. Chem.* 79(17):6783-6791 (2007).
15. He X, Ma J, Jabbari E, The effect of grafting RGD and BMP peptides to a model hydrogel substrate on osteogenic differentiation of bone marrow stromal cells, *Langmuir* 24(21):12508-12516 (2008).
16. Mercado AE, He X, Xu W, Jabbari E, Release characteristics of a model protein from self-assembled succinimide-terminated poly(lactide-co-glycolide ethylene oxide fumarate) nanoparticles, *Nanotechnology* 19(32):325609 (2008).
17. Zhao X, Design of self-assembling surfactant-like peptides and their applications. *Curr. Opin. Colloid Inter. Sci.* 14950 340-348 (2009).
18. Mercado AE, Jabbari E, Peptide-induced self-assembly of synthetic poly(lactide fumarate) macromer, *Trans. Soc. Biomaterials* p. 118 (2009).

19. Hoang QQ, Sicheri F, Howard AJ, Yang DSC, Bone recognition mechanism of porcine osteocalcin from crystal structure. *Nature* 425:977-980 (2003).
20. Sarvestani AS, He X, Jabbari E, The Effect of osteonectin-derived peptide on the viscoelasticity of hydrogel/apatite nanocomposite scaffolds. *Biopolymers* 85(4):370-378 (2007).