Light Wavelengths to Regulate the Release of Multiple Growth Factors

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Statement of Purpose: Localized and timely release of growth factors are critical factors in tissue engineering and regenerative medicine to precisely direct and manipulate tissue growth and differentiation.[1] Most of the existing strategies in controlled release are capable of regulating only single growth factor molecules. Control over multiple growth factor release is of growing importance in tissue engineering to drive the tissue growth toward maturation,[2] since number of natural tissue growth is not simply implicated to only single signaling molecules, but make use of different growth factors as signaling molecules at different stages of their growth in varied ratios. However, design of material strategy that allow precise control over multiple signaling molecules is a challenging task. Here we present sequential and orthogonal control over dual growth factor release from single hydrogel depot, using two different photocleavable molecular systems[3] that undergo light wavelength specific cleavages (Figure 1a).

Methods: All the reagents used in the syntheses were purchased from commercial sources unless otherwise mentioned. Coumarin (CM) and nitrobenzyl (NB) derivatives[3] were utilized in the study as photocleavable units. All the synthetic intermediates and photocleavable units were synthesized and characterized by ^{1}H-NMR and mass spectrometry. 300 MHz Bruker instrument was used to obtain ^{1}H-NMR spectra.

Light wavelength selective cleavages of photocleavable units were characterized by exposing them to 365 and 405 nm (10mW/cm^2) light sources and analyzing the degraded products using high permeation liquid chromatography (HPLC).

Poly(ethylene glycol) (PEG) based hydrogels to covalently contain dual growth factors were produced using azide-alkyne click chemistry. For this, end functionalization of 4-armed PEG polymers with click functionalities (azide and alkyne) was achieved from commercially available polymeric PEGs (M.Wt:-10kDa, Jenkem).

Bone morphogenetic proteins (BMPs): BMP-2 and BMP-7, obtained from Sigma-Aldrich, were used as model growth factors. BMP-2 and BMP-7 were separately tethered to the above established photocleavable units (CM and NB) and then end-functionalized with azide to covalently introduced into click PEG hydrogels. Hydrogels were formulated using 4-armed PEG azide and 4-armed-PEG alkyne to covalently contain both BMP-2, functionalized with NB and BMP-7, functionalized with CM, in hydrogel network. Light controlled BMPs release from hydrogels was monitored using Enzyme-Linked Immunization Assay (ELIZA); Specific ELIZA kits were obtained from Sigma-Aldrich for BMP-2 and BMP-7.

Results: Our HPLC studies on photocleavages of coumarin and nitrobenzyl units utilized in this study exhibited wavelength selective cleavage trend. At 365 nm light, coumarin, CM molecules exhibited higher cleavage rate as compared to nitrobenzyl (NB) derivatives. When studied at 405 nm, the cleavage trend was opposite, i.e., nitrobenzyl showed enhanced cleavage over coumarin units. After establishing the wavelength selective photocleavage, we exploited these cleavable units to independently control the release of two different growth factors. In this regard, BMP-2 and BMP-7 were covalently introduced into PEG hydrogels via these photocleavable units: CM and NB and investigated for light wavelength selective release of BMPs. Light triggered protein release studies indicated increased release of BMP-7, that is attached to CM as compared to BMP-2, when hydrogels exposed to 365 nm light for 4 minutes, clearly suggesting the enhanced cleavage rate of CM at 365nm. But the exposure to 405 nm light for 12 minutes resulted in way higher release of BMP-2, that is tethered to NB, over BMP-7. After studying the independent release of BMP-2 and BMP-7, we also further investigated the possibility of releasing both growth factors one after the other at different time points. To test this, hydrogel was first exposed to 405 nm to selectively release BMP-2. After reaching the plateau in BMP-2 release, when the gel was again exposed to 365nm light, we observed the enhanced release of BMP-7.

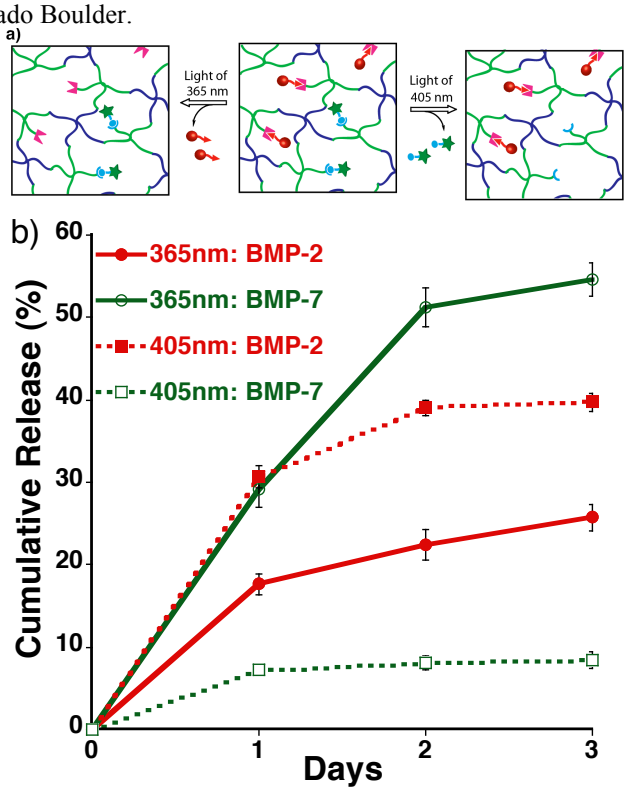

Figure 1. a) Cartoon representation of wavelength selective release of dual growth factors from single hydrogel depot; b) Selective release of BMP-2 and BMP-7 at 365 and 405 nm light exposures from a single hydrogel system that contain both the growth factors.

Conclusions: We have developed two different photocleavable units that can be independently cleaved at light of different wavelengths. We demonstrated the independent cleavage of these molecular units exposing them to 365 and 405 nm light wavelengths and studying the degradation using HPLC. The wavelength selective cleavage of these units was further leveraged to regulate the release two different growth factor molecules. In this study, we have verified not only independent release of BMP-2 and BMP-7 but also their orderly release one by one. We expect that the presented work will open new opportunities for researchers to regulate localized release of different growth factors in a timely manner and eventually will have significant implications in both tissue engineering and regenerative medicine. Further the material design and strategy presented here will bring new insights to design improved materials for controlled release applications.

References:

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