Injectable Pseudoplastic Hydrogels from a Dual-component Dock-and-Lock Self-Assembling Mechanism

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Statement of Purpose: Injectable hydrogels are promising as cell and drug carriers.[1] Current injectable hydrogel systems are often based on the in-situ gelation of injectable liquid precursors; however, this approach can reduce cargo viability and lead to precursor/cargo dispersion prior to gelation.[2] In contrast, pseudoplastic and self-healing hydrogels can be directly injected into tissues and gel locally at physiologic conditions without detrimental stimuli.

To this end, we have engineered self-assembling, pseudoplastic, self-healing, and dual-component hydrogels, exploiting the interaction between cAMP-dependent protein kinase (PKA) and A-kinase anchoring protein (AKAP). One component is a four arm PEG conjugated to peptide ligands derived from the A-kinase anchoring domain (AD) of AKAP.[3] The second component is a dimerization and docking domain polypeptide (DDD) derived from the R subunit of PKA.[4] Gelation occurs when the DDD polypeptide dimerizes and is bound by the multi-arm AD-PEG, using the dock-and-lock mechanism (DnL, Fig. 1A). These interactions are specific and transient, leading to unique hydrogel properties that permit injectability under mild conditions.

Methods: The expression plasmid coding for polypeptide DDD was synthesized by DNA2.0, and DDD was expressed in BL21*DE3 via autoinduction. DDD was purified by immobilized nickel ion chromatography. Four-arm PEG-Maleimide (MW 10kDa) was conjugated with Cysteine-AD to form PEG-AD. DDD and PEG-AD were water dialyzed and lyophilized for subsequent use. DDD and PEG-AD were resuspended to known concentrations separately, and subsequently mixed for gelation. The mechanical properties of gels formed by mixing DDD and PEG-AD in PBS were investigated by cone rheometry. Oscillatory frequency sweep (between 0.01 s^{-1} to 100 s^{-1}), strain sweep (between 0.5% to 500%), and shear recovery (alternating between 0.5% and 500% strain) studies were performed for hydrogels of various wt% compositions (up to 10wt%) at a 2:3 PEG-AD to DDD ratio. Control DDD and unmodified PEG suspensions were also investigated. Human MSCs were suspended with PEG-AD in hMSC culture media, and were mixed with DDD for encapsulation. Cell viability was monitored by confocal microscopy of live-dead stained MSCs. Viability was investigated either 3 hours after gelation or after cells were loaded into a syringe and injected into a collagen gel (via shear-thinning). Injected cells were studied either after 3 hours or after two days of culture.

Results: PEG-AD and DDD rapidly form DnL gels when mixed together, in PBS as well as serum containing media, whereas DDD mixed with unmodified PEG does not gel upon mixing. The physical properties of DnL gels can be tuned by varying gel wt%, and gels with G' over 900 Pa (at 10 wt%) can be formed. The gels have a relaxation time of ~1 s, and behave like viscous liquids at lower oscillatory frequencies. The gels thin when excessive shear strain is applied, and instantaneously self-heal to the starting modulus upon removal of shear strain (Fig 1B). Gels may undergo multiple cycles of shear-thinning and self-healing without losing physical fidelity. Confocal microscopy shows that human MSCs may be encapsulated throughout a 3D gel simply by mixing PEG-AD and DDD containing MSCs and can be cultured with high cell viability (>90%). MSCs encapsulated in these gels may be injected into a collagen scaffold by a syringe, via shear-induced flow and remain highly viable after two days of culture (Fig 1C).

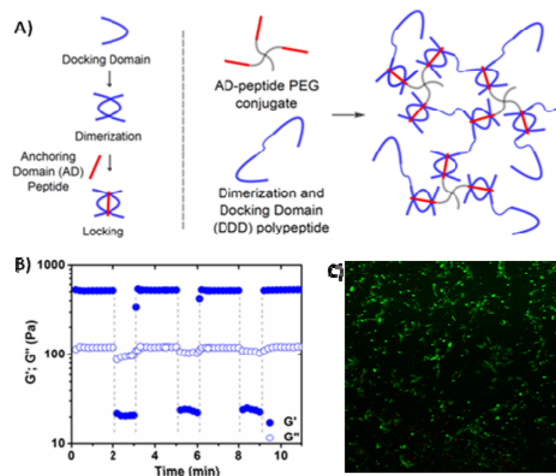

Figure 1. (A) Schematic of the Dual-Component DnL mechanism. PEG-AD and DDD gel upon mixing. (B) Shear-thinning and instantaneous recovery of a 7.5wt% gel. 500% strain thins the gel, which rapidly recovers when the strain is reduced to 0.5%. (C) Confocal imaging of live-dead stained MSCs encapsulated in DnL gels, and injected into a collagen scaffold after 48 hrs of culture.

Conclusions: We have engineered pseudoplastic hydrogels that self-heal, are injectable, and gel at physiological conditions via mixing of two components that exhibit intermolecular interactions. This was accomplished using a self-assembling DnL mechanism, based on forming networks from transient AD and DDD interactions. The injectable DnL gels developed here may be useful for the delivery of cells and other cargo. The gel physical properties are tunable by changing the gel composition, and the moduli of these DnL gels are higher than many other reported shear-thinning gels. The shear-thinning and rapid self-healing properties of DnL gels do not damage encapsulated cells and utilize mild gelation conditions. DnL gels form in serum-containing medium, and are compatible with MSCs, attesting to the high specificity of DnL interactions and their ability to be used as a stem cell delivery vehicle.

References:
[1] Huaping Tan et al. *Materials* 2010, 3, 1746-1767. [2] Qingpu Hou. *J. Mater. Chem.*, 2004, 14, 1915-1923. [3]Mathhew Gold. *Molecular Cell, 2008,* 24, 383–395.

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