Buoyancy-driven Gradients for Biomaterial Fabrication and Tissue Engineering

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Editorial

The controlled fabrication of gradient materials is becoming increasingly important as the next generation of tissue engineering seeks to produce inhomogeneous constructs with physiological complexity. Current strategies for fabricating gradient materials can require highly specialized materials or equipment, and cannot be generally applied to the wide range of systems used for tissue engineering. In this report, the fundamental physical principle of buoyancy was exploited as a generalized approach for generating materials bearing well-defined compositional, mechanical or biochemical gradients. Gradient formation was demonstrated across a range of different materials (e.g. polymers, hydrogels) and cargos (e.g. liposomes, nanoparticles, extracellular vesicles, macromolecules, small molecules). As well as versatility, this buoyancy-driven gradient approach also offers speed (<1 min) and simplicity (a single injection) using standard laboratory apparatus. Moreover, this technique was readily applied to a major target in complex tissue engineering: the osteochondral interface. A bone morphogenetic protein 2 gradient, presented across a gelatin methacryloyl hydrogel laden with human mesenchymal stem cells, was used to locally stimulate osteogenesis and mineralization and produce integrated osteochondral tissue constructs. The versatility and accessibility of this fabrication platform should ensure widespread applicability and provide opportunities to generate other gradient materials or interfacial tissues. In nature, gradients play an essential role in guiding the function of a wide range of tissues, including tendon, cartilage and the central nervous system. Strategies that seek to engineer tissues in vitro must strive to recreate these natural gradients in order to produce fully functional grafts or physiologically-relevant models. In doing so, one must consider the numerous gradients present in native tissue, including transitions in biochemical composition (e.g. extracellular matrix, soluble growth factors) and physical environment (e.g. stiffness, topography).

These transitions play a major role in defining the formation of cellular gradients by spatially regulating the morphology, behavior and differentiation of local cells. Accordingly, intensive research effort has been invested in designing materials with well-defined gradients. For instance, material gradients have been fabricated using multi-channel microfluidic devices or 3D printing. However, these approaches require specialist apparatus and are usually restricted by certain material parameters, such as viscosity or gelation kinetics. Approaches such as photopatterning and magnetic field alignment have also been used to generate gradients; however, these remote field strategies require even more specific material properties (photoresponsivity, magnetic susceptibility). Indeed, a generalized method that can be universally applied to different systems has thus far remained elusive. In this work, we sought to employ a more universal physical principle that could be applied broadly across different material systems. Specifically, we investigated whether we could fabricate material transitions using buoyancy, the upward force generated on materials immersed in a denser fluid phase. Buoyancy is commonly used to stabilize the formation of sucrose gradients used for the fractionation of cells and organelles. This approach has been extended to nanoparticle separation using density-graded organic solvents. There are only limited examples in which buoyancy has been used to fabricate gradient materials. For example, Parameswaran and Shukla described a system in which cenospheres (hollow silica-alumina microparticles) were allowed to rise to the top of a polyester resin, and then immobilized in a solid matrix using a 48 h curing process. This yielded a polymeric material with a graded volume fraction of cenospheres, and a corresponding gradient of compressive and tensile properties. Similarly, Beals and Thompson showed that the injection of a gas into a molten aluminum alloy could be dispersed into small particulate-stabilized bubbles upon mixing. These bubbles formed liquid foam at the top of the melt, which was subsequently cooled to form a metallic structure with a gradient of porosity. However, these examples are highly-specific with a focus on solid materials for industrial applications. To the best of our knowledge, there has been no report of any versatile methodology using buoyancy in gradient material fabrication, or any examples in which buoyancy-driven gradients have been applied to the field of complex tissue engineering.

To this end, we sought to develop a generalized buoyancy-driven approach for casting gradients that could be applied to real-world tissue engineering applications. Here we show that a single injection event of one fluid material into another is sufficient to generate material transitions that can be preserved by subsequent gelation or polymerization. Moreover, we were able to fabricate materials exhibiting either a sharp transition or a smooth gradient by systematically varying the material characteristics and injection parameters. We used this platform to cast gradients in several different materials (gelatin methacryloyl, gellan gum, agarose, acrylate polymers) and generated tunable transitions in composition, biochemical profile and compressive stiffness. We also demonstrated that several cargo species could be incorporated in gradient form, including inorganic nanoparticles, liposomes, cell-derived extracellular vesicles, macromolecules and proteins. We applied this method to cast bone morphogenetic protein 2 (BMP-2) gradients for the in vitro engineering of osteochondral tissue constructs. In this system, the encapsulated BMP-2 was slowly released over 28 d of tissue engineering to locally stimulate osteogenesis of human mesenchymal stem cells and produce osteochondral tissue bearing a defined mineral cap. The versatility and simplicity of this gradient casting platform should enable a range of applications in complex material fabrication and interfacial tissue engineering.

In order to rapidly cast different material gradients, we sought to develop and optimize a controlled two-component mixing system. Specifically, we used a commercially-available electronic autopipette to introduce one material at a defined rate into another static base material. The two phases were allowed time to establish a gradient, which could then be preserved by triggering a polymerization or gelation process. To characterize this system and elucidate the mechanism behind gradient formation, we analyzed the composition of the hydrogel along the longitudinal axis. We divided the hydrogels into four transverse sections, which we analyzed using an enzymatic assay for sucrose and fluorescence spectroscopy for dye-labelled agarose. These measurements revealed a sucrose gradient within the hydrogel that was inversely associated with the injected dye-labelled agarose. These results suggest that sucrose must retain attractive intermolecular interactions with the base layer agarose during the fluid-mixing process.

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