Bijels-derived Hybrid Hydrogel Membranes and Growth Factor Delivery

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Introduction: Bicontinuous interfacially jammed emulsion gels ("bijels") are a new class of materials consisting of two interpenetrating continuous phases, each being filled with a liquid that is immiscible with the other [Cates ME and Clegg BS, *Soft Matter*, 2008, 4:2132-2138]. The unique bicontinuous phase morphologies and other properties of bijels present bijels and also bijels-derived materials for many potential applications, including microreactors, fuel cells, and tissue engineering. Bijels can be made by several techniques and solvent transfer-induced phase separation (STRIPS) is attractive as it enables continuous bijels fabrication and can produce bijels particles, fibers and films [Haase MF, *et al.*, *Adv Mater*, 2015, 27:7065-7071]. Using the templating approach, co-continuous Ni/Ni$(OH)_2$ porous electrodes have been made [Witt JA, et al., *J MaterChem A*, 2016, **4**:1000-07]. The fabrication and characteristics of bijels-derived membranes for biomedical applications should also be investigated. The hydrogel phase in bijels-derived materials may encapsulate bioactive molecules, such as growth factors (GFs), making them promising vehicles for controlled release in tissue engineering. Vascular endothelial growth factor (VEGF) plays a vital role in angiogenesis and can influence the survival, migration and proliferation of endothelial cells. This study thus investigated VEGF-containing bijels-derived materials.

Methods: STRIPS was modified for producing bijels in this study. Briefly, a ternary liquid mixture was prepared by adding ethanol, hexanedioldiacrylate (HDA), 2-hydroxy-2-methylpropiophenone, deionized water, Ludox TMA, and cetyltrimethylammonium bromide (CTAB) in ethanol (0.2M). The mixture was adjusted to pH3 by adding HCl. A polystyrene plate was immersed in the ternary mixture for forming a mixture film on its surface. It was taken out and then immersed in a water bath (1×10^{-3}M CTAB) to form bijels membranes. A high-intensity UV light (wavelength: 340 nm) was used to cure HDA, solidifying the bijels structure. After UV curing, bijels membranes were immersed in a Na-alginate solution or a VEGF-containing Na-alginate solution, taken out and immersed in a $CaCl_2$ solution for crosslinking Na-alginate into Ca-alginate. Finally, the products were dried. Structure of bijels membranes before and after introducing Na-alginate were studied using SEM. Fibroblasts (NIH 3T3) were cultured on bijels-derived membranes whose biocompatibility was assessed using LIVE/DEAD cell viability tests. MTT assay was used to evaluate cell proliferation on bijels-derived membranes, and cell morphologies were examined at different time points of cell culture. Following our established test protocol, the *in vitro* release behavior of VEGF for bijels-derived membranes was studied.

Results: For bijels and bijels-derived membranes, bicontinuous phase morphologies were clearly seen. In Fig.1a, the continuous solid component was UV-cured HDA and the pores were spaces occupied by the water phase in bijels that had evaporated. After introducing Na-alginate into bijels to form bijels-derived membranes, the contours of two phases (HDA and alginate) in membranes were distinguishable. The results indicated the successful fabrication of bijels and subsequently hybrid hydrogel membranes via bijels. Compared to other bijels fabrication techniques, STRIPS has distinctive advantages due to its feasibility for a broad range of raw materials and also the bijels morphologies that it can generate. The current investigation suggests the need to explore STRIPS-formed bijels and bijels-derived materials for biomedical applications. In the VEGF release experiments, slight initial quick release was noted. This may be caused by the VEGF molecules on the surface of bijels-derived membranes. A steady and sustained release of VEGF was seen in the remaining test duration (Fig.1b). For *in vitro* biological investigations, fibroblasts were cultured on bijels-derived membranes for different times up to 3 days. LIVE/DEAD cell viability tests conducted at different time points revealed good biocompatibility of these membranes. Fibroblasts proliferated on the membranes and nearly no cell death was observed (Fig.2a). Cell proliferation was quantified and the MTT results showed good cell proliferation after 1, 2, 3-day culture on bijels-derived membranes (Fig.2b).

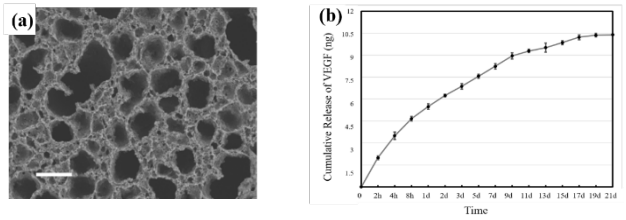

Fig. 1. (a) Bijels formed by modified STRIPS, (b) *In vitro* release behavior of VEGF from bijels-derived membranes

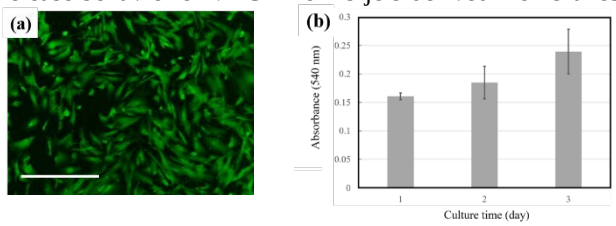

Fig. 2. *In vitro* biological studies of bijels-derived membranes: (a) Cell viability, (b) Cell proliferation

Conclusions: The exploration on bijels and bijels-derived hybrid hydrogel membranes for biomedical applications was started. The STRIPS technique with some modifications appeared suitable for fabricating bijels and bijels-derived hydrogel membranes. GF could be encapsulated in the hydrogel phase of bijels-derived membranes and it exhibited steady and sustained *in vitro* release. *In vitro* biological investigations showed that the bijels-derived membranes were biocompatible and that cells could proliferate well. This study has demonstrated the potential of bijels technology in the biomedical field.