Designer Gels for Cell Culture and Bioprinting Applications

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Biogelx designs and supplies tuneable peptide hydrogels, offering artificial tissue environments for a range of cell culture applications. These highly tuneable, cell-matched biomaterials are three dimensional (3D), 99% water and have the same nanoscale matrix structure as human tissue, tuned to meet the needs of any given cell type. The ability to precisely control the mechanical and chemical properties of these hydrogels is creating new opportunities in the fields of stem cell research, 3D cell culture, cell-based assays, cancer cell research, regenerative medicine, and bioprinting.

Hydrogels Tuned to Match Tissue Environments

The chemical and physical properties of the hydrogels can be tailored to meet the needs of specific cell types, thereby enabling the study and manipulation of cells in a more realistic (in vivo-like) 3D environment that is **simple, fully defined and tuneable**. The products unique cell-matching capabilities clearly provide academic users, medical researchers and pharmaceutical companies with a serious alternative to competing 3D cell culture products.

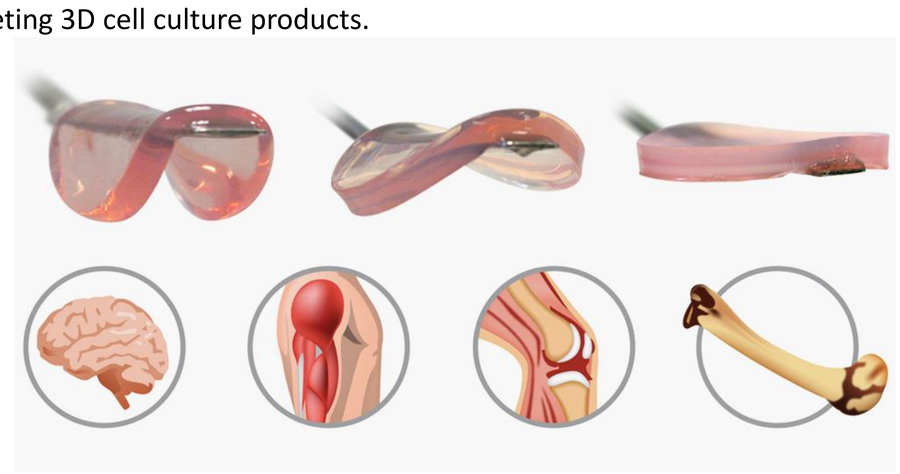

Figure 1: Example of some of Biogelx's 3D *in vitro* hydrogels, tuned to match different tissue environments.

The Technology – Self-assembling Peptides

Biogelx's hydrogels are based on the combination of Fmoc-diphenylalanine (Fmoc-FF) and Fmoc-Serine (Fmoc-S) peptides. Fmoc-FF alone self-assembles to form fibers in aqueous environments (gelator peptide), whilst Fmoc-S would form micellular structures by comparison (surfactant peptide). By combining the two peptides, it produces fibers of Fmoc-FF coated with Fmoc-S, which presents hydroxyl functionality on the surface of the fibers ('core-shell' assembly), and thus presenting a suitable surface chemistry for cell adhesion (**Figure 2**).

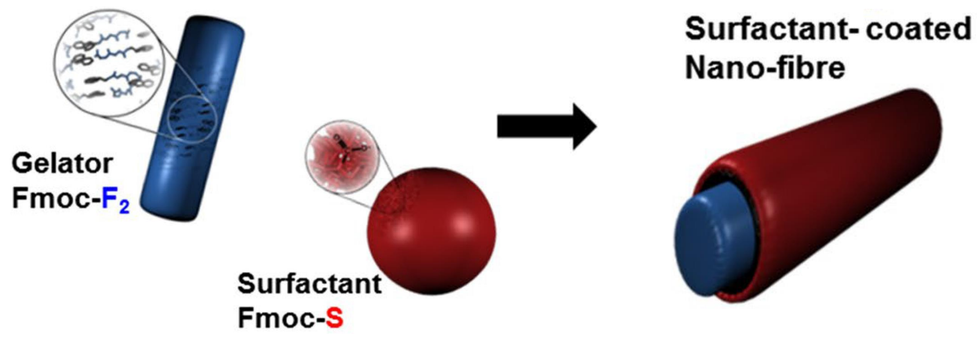

Figure 2: Schematic representation for the self-assembly of Fmoc-di-Phenylalanine (blue), Fmoc-Serine (red) and combined Fmoc-FF/S peptides in aqueous environments.

Flexible Handling

The gels are applicable across a range of cell-based applications. Gelation is triggered through addition of cell culture media (**Figure 3**), offering a flexible approach, where cells can be cultured inside the gel (3D culture) or on top (2D culture).

Figure 3: Gels are supplied as a lyophilized peptide powder and rehydrated with water to achieve pre-gel solution. Then, addition of cell media encourages fibers to lock, giving desired gel stiffness.

Dictating Stem Cell Differentiation Using Hydrogel Stiffness

Specifically, these hydrogels have been shown to aid stem cell migration, differentiation, survival and integration. **Figure 4** presents results from growing mesenchymal stem cells on three Fmoc-FF/S hydrogels of varying stiffness, but identical peptide chemistry. Each hydrogel was found to direct differentiation of the cells into specific lineages, as they presented bone (rigid gel), cartilage (stiff gel), or neural (soft gel) cell phenotypes in the absence of differentiation media, matrix proteins or bioactive motifs.

This data highlights the influence a highly tuned 3D environment can have, in the context of stem cell differentiation.

Figure 4: Example of how stiffness alone influences the differentiation pathway for human mesenchymal stem cells. β3-tubulin, neuronal marker; SOX-9, chondrocyte differentiation marker; RUNX-2, osteoblast differentiation marker.

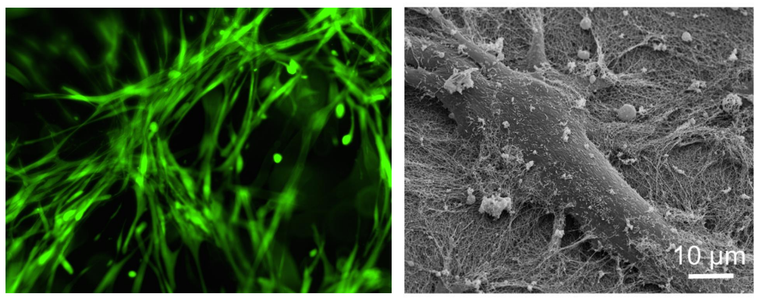

Figure 5. Example of human MSCs cultured in a rigid gel and stained with calcein AM (*left*). SEM image of a single MSC attached to Fmoc-FF/S gel fibers (*right*)

3D Bioprinting

Biogelx are optimising protocols that adapt these hydrogels for use in 3D bioprinting. It is known that salts in the cell media are the trigger for gelation in standard cell culture methods, as such this concept can be applied to control gelation to suit 3D Bioprinting methods.

Varying ratios of pre-gel and cell media allows constructs of good strength to be generated. **Figure 6** shows structures created by printing layers of Biogelx gel.

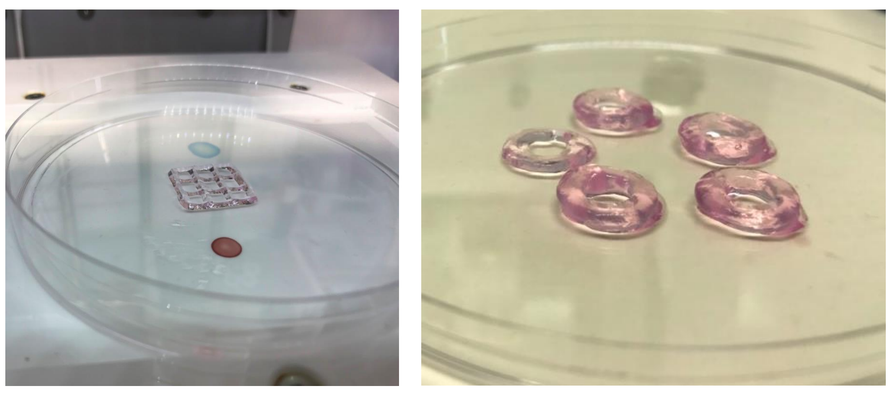

Figure 6. Photograph of structures produced by extrusion printing using Biogelx gel: a grid (left) and a ring structure (right).

REFERENCES

- Alakpa *et al., Sci. Rep,* **2017**, *7:* 6895
- Alakpa *et al., Chem,* **2016**, *1:* 298-319
- Jayawarna *et al.*, *Acta Biomaterialia*, **2009**, *5:* 934 – 943
- Zhou *et al.*, *Biomaterials*, **2009**, *30:* 2523 – 2530
- Smith *et al.*, *Adv. Mater.*, **2008**, *20:* 37 – 38