Biogelx: Designer Gels for Cell Culture

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ABSTRACT

Biogelx is an early stage biomaterials company which designs and supplies peptide hydrogels for cell culture, precisely tuned to the requirements of the cell. These innovative cell-matched gels enable replication of the natural environment of cells, in the artificial environment of the laboratory. These products have applications in cancer modelling research, toxicology assays, 3D bio-printing, and may ultimately be translated to clinical and regenerative medicine. The chemistry and physical properties of the hydrogels match the characteristics experienced by cells within human tissues, thus enabling the study and manipulation of cells in a realistic 3D environment which is simple, fully defined and tunable.

Keywords: biomaterials, hydrogels, cell culture, bioprinting, drug delivery

1 COMPANY OVERVIEW

Biogelx Limited is a biomaterials company that designs tuneable peptide hydrogels, offering artificial tissue environments to cell biologists for a range of cell culture applications.

This technology is capable of revolutionising the way cell biologists control and manipulate cell behaviour in the laboratory. This is of direct relevance to fundamental cell research, including the study of stem cells and disease models within academic and medical labs. However, the major commercial significance this has is the dramatic impact on the development of cell-based assays and drug discovery/toxicology platforms within large pharmaceutical companies, representing a rapidly growing global market with revenues of over $5 billion per annum.

The Company was founded in December 2012. To date the company has launched a commercial research grade product, which is selling across four continents to a range of customers including academic biologists from leading institutions, SME’s and major pharmaceutical companies. Predominantly, the gels are used in applications associated with *in vitro*, lab-based research at the ‘well plate’ scale.

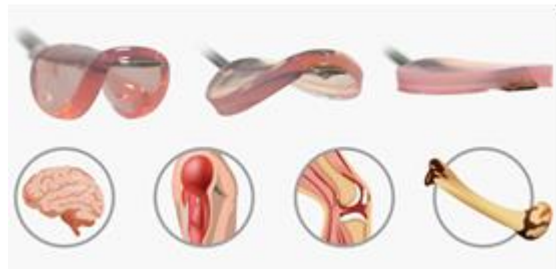

Figure 1: Example images of some of Biogelx’s 3D in vitro hydrogels, tuned to match different tissue type environments.

2 PRODUCT DESCRIPTION

Biogelx offers a range of hydrogel platforms that are three dimensional (3D), 99% water and have the same nanoscale matrix structure as human tissue (Figure 1).

The technology is based on the combination of short peptides that self-assemble and form fibers in aqueous environments. These fibers present a suitable surface chemistry for cell adhesion. In designing these products for cell culture applications, the mechanical properties and ultimately stiffness of the gels can be manipulated through variations in peptide concentration and other additive factors.

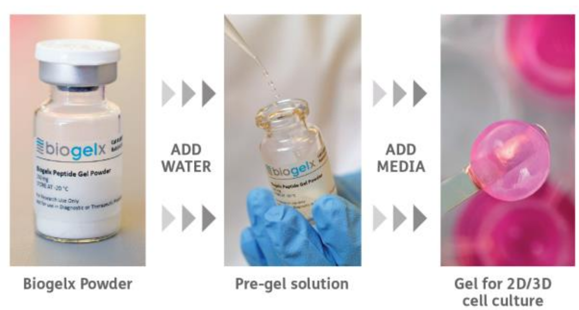

Figure 2: Gels are supplied as a lyophilized peptide powder and rehydrated with water to achieve pre-gel solution. Then, addition of cell media encourages fibers to lock, giving desired gel stiffness.

Biogelx supplies its products as lyophilized powders. Simple rehydration with water achieves a pre-gel, free flowing solution. The fibers form the gel when they come in contact with the calcium present in cell culture media. Calcium acts as a cross linker, locking the fibers in place to create the scaffold.

2.1 Technology background

The core technology that is embedded in the company's commercial products is that of self-assembling peptide amphiphiles.

Aromatic peptide amphiphiles contain a short (e.g. di- or tri-) peptide sequence, with the N-terminus capped by a synthetic aromatic moiety. Such compounds are commonly utilised in the preparation of supramolecular hydrogels. In these cases, self-assembly is based upon aromatic stacking interactions, and the propensity of the peptide to form a β-sheet type Hydrogen bonding arrangement.

The underlying basis of Biogelx's gel technology is the ability to strategically synthesise and combine different types of aromatic peptide amphiphiles. The selection of peptide sequence and aromatic ligand determines gelation capability, together with the nanoscale environment experienced by the cultured cells.

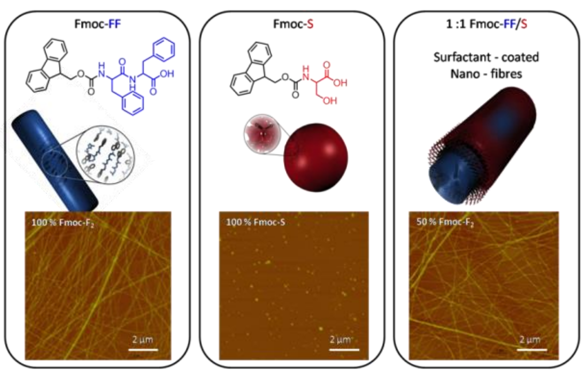

Figure 3: Schematic representation of the self-assembly of Fmoc-FF (blue) forming fibres, Fmoc-S (red) forming micelles, and a 1:1 ratio of Fmoc-FF:Fmoc-S forming fibres coated with a surfactant.

Biogelx's standard hydrogel technology is based on the combination of Fmoc-diphenylalanine (Fmoc-FF) and Fmoc-Serine (Fmoc-S) peptides. Fmoc-FF, a hydrophobic dipeptide, is well known to self-assemble and form fibres in aqueous environments induced by changes in pH (figure 2). Since serine (S) has a hydrophillic side chain (-OH), it is presumed that the aggregation is to minimise contact of the aqueous solvent with the hydrophobic Fmoc group. By combining the two starting materials and inducing self-assembly through pH change, fibres of Fmoc-FF, coated with Fmoc-S (acting as a surfactant) are produced. These fibres present hydroxyl functionality on the surface ('core-shell' assembly), thereby presenting a suitable surface chemistry for cell adhesion (known as the Fmoc-FF/S system).

In designing these products for *in vitro* cell culture applications, the mechanical properties and ultimately stiffness of the gels, can be manipulated through variations in peptide concentration and other additive factors.

In cell-based applications, the fibres only form a gel when they come in contact with the calcium ions present in cell culture media. These ions act as a cross linker between surface serine groups, lock the fibres in place and create the scaffold.

Biogelx supplies its products as lyophilised powders, allowing customers to simply rehydrate with water to achieve the pre-gel, free flowing solution. Addition of calcium ions/cell culture media then creates the gel.

This use of lyopholised powder offers many benefits to end users through:

- Enhancing product shelf life (stable for over 1 year if stored at -20 °C).
- Allowing gel stiffness modification, simply through changing the powder volume.
- Providing simple incorporation of components such as proteins, growth factors, to benefit cell growth/viability.

3 APPLICATIONS

Biogelx innovating technology is revolutionizing the way cell biologists control cell behavior in the laboratory. Our gels can be tuned to mimic specifically the environment in which the cells grow in vivo, allowing them to thrive.

This is of direct relevance to several applications:

- Cell culture in fundamental **laboratory research**.
- Harnessing the capabilities of **3D bio-printing**.
- Development of new **drug discovery/toxicology** platforms within large pharmaceutical companies.
- Clinical and **regenerative medicine**; tissue reconstruction and stem cell research.

3.1 Cell biology research

Cell biology research is the study of the structure, properties, the life cycle, division and death of cells. Knowing the components of cells and how cells work is fundamental to all biological sciences. The challenge facing cell biology researchers is creating an environment in the laboratory that replicates a cell's natural environment and allows it to behave in a realistic manner. It is increasingly understood that one major element that needs to be achieved when studying cell behaviour is the need to create the 3D environment that cells are accustomed to in nature.

In the academic community, bioengineers and cell biologists are working collaboratively to add further complexity and better mimics of that 3D environment beyond 'well plate scale' cell culture through 3D bioprinting. Where they are attempting to build tissue models using single and multiple cell types for a wide range of disease models.

3.2 3D bioprinting

Biogelx hydrogels have a potentially dramatic impact on harnessing the capabilities of 3D bio-printing; a key tool in achieving truly controlled and well-defined tissue constructs.

3D bioprinting utilises 3D printing technology to produce functional miniaturised tissue constructs from biocompatible materials, cells and supporting components such as cell media. Major applications include use in high-throughput in vitro tissues models for research, drug discovery, and toxicology, in addition to regenerative medicine/tissue engineering applications. It involves layer-by-layer, precise positioning of the biomaterials and living cells, with spatial control of the placement of functional components. The technology has made significant progress towards the clinical restoration of tissues and even organs such as ear, nose, bone, and skins (Figure 3).

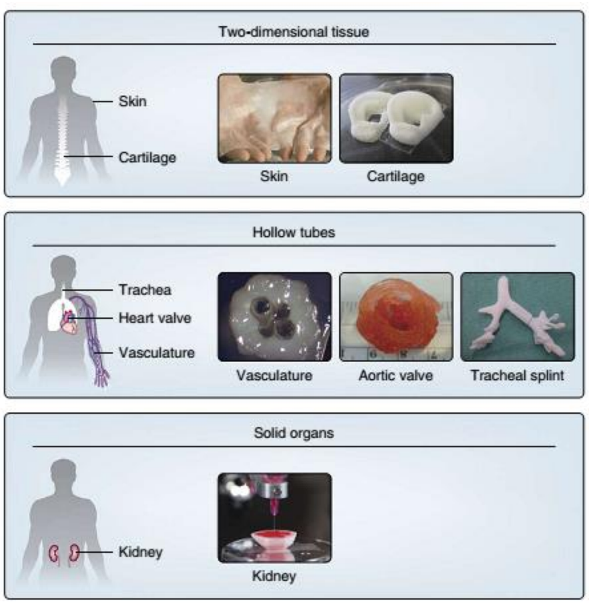

Figure 4: Examples of human-scale bioprinted tissue (adapted from reference 4)

A vital yet limiting component in effective 3D printing of cells, and ultimately miniaturised tissues, is a scaffold material (essentially 'printer ink') to form the 3D structure to support the cells. Such a material must offer properties suited to both the 3D printer mechanism and to hosting/ maintaining the viability of the cells within the scaffold structure. Obtaining such a balance of properties is the main challenge within 3D bioprinting, and is reliant on a number of key features.

Bioink Requirement	Biogelx Solution
Printability	**Synthetic, simple, short peptide chains** allow for well controlled gelation, and careful tuning of viscosity and rheological properties. This should aid compatibility with printer components, in addition to stability of the printed structure.
Biocompatibility	Biogelx hydrogels are **composed of amino acids familiar to the body**, and thus should not illicit any undesired effects.
Structural and Mechanical properties	**Biogelx technology can be tuned to mimic a variety of tissues types**, and therefore the mechanical and structural properties can be tuned to match the cell-type required.
Degradation profile	Hydrogels composed of short, simple peptide chains, mean simple breakdown products.
Biomimicry	Biogelx can successfully **incorporate specific amino acids, including protein-related sequences, into its hydrogels** to help tune the chemical environment towards specific cell types. This includes RGD peptide from fibronectin or sequences from laminin to aid cell attachment.

Table 1: Innovative features/advantages of Biogelx hydrogels.

Biogelx hydrogel technology aligns very well with this need for a compatible ink to build 3D tissue constructs with cells.

3.3 Cell-Based Assays - Drug Discovery

Cell-based assays refer to the use of live cells in the study of the effects of various chemicals on the body, as opposed to more traditional biochemical methods, which do not involve the use of cells. It is recognised that cell-based assays have the potential to offer a more accurate representation of the real-life model, particularly if this is carried out in a 3-D manner.

There is a growing trend towards more frequent use of cell-based assays for drug discovery, especially since the toxic response of cells is fundamental to the successful development of new drugs by ‘Big Pharma’. Reports have highlighted that the rapidly growing cell-based assay market is worth over $6 billion per annum, with an annual growth of over 10% (Cell-Based Assays: BCC Research 2015).

3.4 Cell Therapies/Regenerative Medicine

Cell therapies are treatments involving the introduction of new cells into a tissue in order to address a disease (e.g. diabetes, stroke, spinal injuries). Regenerative medicine is one facet of this work. Cell therapy is recognised as a potentially disruptive technology; however, it is not new. It has its origins in blood transfusion, bone marrow and organ transplantation, tissue banking and reproductive in vitro fertilization.

Recent reports have highlighted a global market size of over $25 billion per annum in 2015, with an annual growth of over 20% and which the US makes up 50% of. It has been further identified that hydrogel technologies in this market make up $3 billion of it, and therefore represents the addressable market for Biogelx (Global Tissue Engineering and Regeneration: BCC Research August 2014)].

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