Engineered living materials (May 4–7, 2021)

Enateri V. Alakpa

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The second virtual conference of Engineered Living Materials took place on May 4–7, 2021. The conference aimed to bridge material science, biophysics, and synthetic biology to create composite materials constituting living cells augmented with non-living matter. These novel innovations have broad-based applications, such as textiles, mass manufacturing, and clinical therapeutics. Living materials hold vast potential for programmable functions and is a promising means of shaping the ways we approach the design and fabrication of clinically relevant devices. This News in brief highlights some of the presentations that the EBioMedicine readership could also find of interest.

1. Versatile living materials fabrication by integrating engineered microbes and polymers

In the session titled Bioprocessing functions, Zhuojun Dai (Shenzhen Institute of Synthetic Biology, Shenzhen, China) presented work from her group with engineered bacterial cells (eg, Escherichia coli), producing a target protein, are combined with a synthetic hydrogel to create a living functional material. In Bioprocessing, an ePOP circuit is created by genetically altering bacterial colonies to produce a particular protein or molecule. When a plasmid is overexpressed, the cell subsequently dies. On reaching a high enough cell density, cell death occurs en masse (popping), a process which can be clearly seen via microscope. Dai and colleagues make use of this type of circuit to facilitate synthetic biology in creating functional materials.

Encapsulating this synthetic circuit with a hydrogel as the primary scaffold means that the target protein or molecule produced by the bacterial cells can be immobilised within the hydrogel material, leading to the formation of a new material which Dai and colleagues had termed a semi-interpenetrating polymer network (sIPN).

A clear example of the potential application of this material was for the protection of the gut microbiome, which the group had tested by exploring the material performance after antibiotic perturbation in mice. This experiment was done over 2 weeks and compared against a known gut shield, beta-lactamase, and a non-composite hydrogel. In all cases, they observed that the living sIPN was the most efficient at maintaining gut microbiota stability when exposed to antibiotic damage.

Aside from the gut microbiome protection, the technique holds the potential for an array of material design to facilitate drug delivery.

2. Combining cells and hydrogels for on-demand production capabilities

The Alper Laboratory based at the University of Texas (TX, USA), in collaboration with Alshakim Nelson’s group at University of Washington (WA, USA) explored the possibility of creating practical methods that aid the scale-up of molecule biomannufacturing with a focus on high demand compounds such as ethanol. In this pursuit, Professor Alper presented how the synergy between cells and hydrogels could be best exploited, focusing on cell embedding, preservation, and functional output (the production of specific biomolecules, eg ethanol).

Using saccharomyces cerevisiae as a model organism, they make use of F127-bisurethane methacrylate (F127-BUM) hydrogels as a scaffold. This hydrogel is of particular advantage as its temperature dependent solution to gel transition allows even distribution of the cells throughout the hydrogel. It possesses shear thinning properties that allows for extrusion printing of the cell-gel mixture. A particularly interesting facet of the F127-BUM hydrogel is its ability to protect both E coli and yeast cells against damage from lyophilisation, which is advantageous with regards to long-term storage. Within this system, Alper and colleagues observed continued yeast function in both the preserved and non-preserved gels, acquiring a steady output of ethanol for approximately a year. Compared with liquid culture systems, the gel cultures consistently exhibited better stable protein activity and significantly higher production quantities, irrespective of whether the gels were immediately cultured or lyophilised for preservation.

Going further, the group showed how the system could be diversified by using engineered E coli (producing the small molecule 2,3 betanediol) and yeast cells (producing the small molecule L-Dopa) as well as the production of larger proteins (eg, colicin V) from E coli. The physical properties of the hydrogel also have an important role by enabling compartmentalisation during co-culture to avoid competing bacterial growth. Alper and colleagues were able to enforce...
spatial restriction during co-culture, allowing control of the endpoint dynamics of the culture system that would otherwise be extremely difficult for two or more cell types that have contrasting properties or behaviours.

3. Building tissues cell by cell using light

In the living therapeutic materials session, Seraphine Wegner (University of Münster, Münster, Germany) presented research by her group based in which they explored the induction of cell-cell adhesions using light. From this starting point, the group was able to investigate factors that were particularly important for early tissue organogenesis and development.

Breast cancer cells MDA-MB-231 that produce genetically encoded switchable surface proteins (eg, Cry2 and CIBN) allow for protein complex formation under non-toxic, low intensity blue light, leading to cellular aggregation. This reaction is reversible in the dark, allowing the interaction to be switched on and off repeatedly. Through this principle, Wegner and colleagues were able to produce cells that associate and dissociate when exposed to differing wavelengths of light (blue, red, and far red). The aggregation patterns of these cells were dependent on the protein type and dynamic control of kinetic and thermodynamic parameters, shown by the difference noted when the cells were exposed to continuous or pulsed light.

Wegner and colleagues then showed that in addition to controlled cell-cell adhesion, cellular dynamics is also a crucial factor in the development of multicellular structures. By establishing photoswitchable cells with varying binding strengths, protein–protein interaction dynamics, and reversion kinetics, they were able to control the spatial arrangement of specific cell types such that they mimic the specific arrangement of a blastocyst, for example.

This technique, which facilitates a bottom-up approach to engineering tissue types, holds particular promise for furthering our understanding of innate tissue functions and for modelling in vitro disease states due to early perturbations in development.

4. Cell-rich bioengineered devices for regenerative medicine

Another bottom-up strategy for engineering tissue constructs was also presented by Professor João Mano from the University of Aveiro (Aveiro, Portugal), who showed examples of how using cell rich constructs can be used to build materials with minimal scaffolding (or completely free of scaffolds).

Inspired by diatom (single celled algae which produce silica cell walls) biominalisation systems – conversion of soluble silica into amorphous silica structures called frustules which play an important role in cell-cell and cell- material adherence, Mano and colleagues showed how individual cells that are partially coated with an adhesive consisting of a chitosan-L-carnitine primer followed by silica deposition could be an advantageous tool for directed cell assembly. Silica coating was done using adherent cells on tissue culture plastic and therefore, on detachment, only the topside (approximately 57%) of the cell is coated, leading to the term silica backpack. An assessment of human derived adipose stem cells (hASCs) in suspension showed that the cells with the silica backpack had good viability, exhibited significantly higher overall metabolism, and formed larger cell aggregates than non-coated cells.

Such cellular aggregation allowed for the formation of scaffold free fibres, an alternative to the more common spherical or sheet aggregation formed in vitro. At high cell density, the fibres form rapidly and in the absence of exogenous materials or supplements. The group was also able to show that these fibre constructs (again using hASCs) were not only a physical construct, but as a living material had trophic, provascular, and immunomodulatory effects when in co-culture with endothelial cells.

The use of magnetic nanoparticles to enforce structured cell sheet assembly was another interesting example of the research done by the laboratory. Iron oxide nanoparticles internalised within hASCs and human umbilical vein endothelial cells (HUVECs) were used to create a stratified magnetic responsive cell sheet assembled layer by layer. By altering the cell numbers, this magnetic force assembly allowed for the construction of multicellular layers with controlled thickness. With this technique Mano and colleagues were able to discern the osteogenic potential of stem cells when cultured as uniform or heterotypic cell sheets. Co-cultures of HUVECs and hASCs showed that this synergistic effect led to overall higher osteogenic activity compared with homogenous ASC cell sheet structures.

Such material minimalists to tissue engineering show particular potential in redressing an imbalance in tissue regeneration when properties such as material integration or degradation can impede or prove challenging to healing progression to traumatised tissue.

Declaration of Competing Interest

None.