Adult stem cell coatings for regenerative medicine

Stem cells can become potent tools for the treatment of degenerative disorders such as heart failure, eye disease and osteoarthritis. Housing stem cells inside a hydrogel coating, directly deposited around them individually and in groups, may be an important solution to the problem of increasing stem cell viability and protection in cultivation. Such coatings can target regulatory proteins and genes for maintenance, differentiation and development into tissues. Already polymer coatings are being applied directly to protect insulin producing pancreatic islet cells in the hope of treating type I diabetes. Here, we review current emerging developments in adult mesenchymal stem cell nanocoating and microcoating techniques and assess their unique practical engineering, biological and potential clinical advantages.

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Regenerative medicine is the application of science and technology to growing fresh, entirely new tissues and organs outside the body from the patient's own cells, especially antecedant stem cells. The ultimate goal is to replace any tissue that is damaged beyond repair, as a result of degenerative disease, genetic defects, and trauma. Medical healthcare will be revolutionized when tissue engineers can generate fresh tissues and new organs rapidly on demand, tailored to each patient by age, disease status, and immunological variance. However, so far the only commercially available full tissue replacements (living tissue with a resorbable carrier material) are skin and cartilage because they are the only ones that can be grown outside the body, in sufficient amounts, and with clinical quality, to properly treat burns and certain skeletal defects[1,2,3].

The reasons behind the low number of replacements for other tissues in the body are: the best type of therapeutic stem cells are not known; insufficient numbers of stem cells can be isolated from a single patient; not enough stem cells can be kept alive in cultivation; and the tissues become damaged during transplantation. Neither is the fate of stem cells implanted inside the body properly controlled. These facts highlight the need for better practices and procedures in stem cell cultivation and targeted placement. Increasingly, there is also a need for better materials with which to process and guide these cells into functional tissues.

In this article we first focus on the utility of biomaterials for regenerative medicine and cell therapy, as they can play the pivotal role in controlling cell development, maintaining cell viability in culture, and protecting cells for transplantation and targeted deployment.

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One approach to sorting out these problems is to accurately recreate the stem cell niche using biomaterial analogues of the natural extracellular matrix. At each stage of stem cell bioprocessing, which involves isolation from tissues, selection from mixtures of cell types, cultivation in tissue culture, and transplantation at targeted localities, biomaterials have a vital role to play[4,5]. The use of biomaterials to facilitate stem cell functions, such as the control of differentiation, may not be mandatory however. Induced pluripotent stem cells can now be produced by transducing selected sets of genes with retroviruses in specialized cells and mesenchymal stem cells[6]. Biomaterials may provide an effective non-viral alternative for the transfection of human cells using gene sets for induced pluripotency.

As we shall highlight in this article, biomaterials of synthetic and natural origin, directly coated onto the cell membrane have the potential to collectively facilitate the stem cell through all these stages, effectively and safely, as the natural stem cell microenvironment is thought to do. We begin this review article by describing the role of microenvironment design on control and regulation of stem cells followed by a description of man-made biomaterial equivalents for these environments. We then describe the two main types of coating micronevironment that have been devised for pancreatic islet cell therapy-micrometric coatings and nanometric coatings. We then describe the few studies that have used the coating principle to encase mesenchymal stem cells for future potential in therapeutics and regenerative medicine. Finally we describe our preliminary work in this area, where we have coated mesenchymal stromal cells with micrometric layers of polysaccharides using molecular interconnectors to cell membrane proteins.

Control of stem cells through the local microenvironment

Considerable evidence points to the existence of specialized privileged microenvironments where reservoirs of stem cells are permanently pooled as a way of maintaining their unique intrinsic properties[7]. They remain there for their own protection in a state of quiescence until they are mobilized into action for routine maintenance, during injury and extensive replenishment for tissue regeneration[8]. The significance of specialized microenvironments on stem cell characteristics is highlighted by the behavior of embryonic stem cells when they are injected into mouse fat tissue[9]. In this new environment they specialize and become uncontrollable, forming tumor masses, but when injected into the sphere of cells of the early stage human embryo they react normally.

Stem cells with the greatest vitality are those that exist in the fertilized egg; the mass of pluripotent embryonic cells enclosed by a protective shell of support cells[10]. Not all stem cells have elaborately privileged compartments being attached in isolation to a basal lamina. The unique stem cell residences are composed of supporting cells a delimiting basement membrane compartment made from extracellular matrix components and retained soluble regulatory molecules[11,12].

Stem cells are also influenced by surrounding tissues and at a higher and remote level by systemic immunological and neuroendocrine signalling[12]. Therefore, to harness stem cells properly for protection, development, and transplantation they have to be given very well defined microenvironments that replicate their native three-dimensional environment composed of extracellular matrices (ECMs). The ECM influences all normal stem cell activities such as movement, development, repair, and regeneration. This is because it is secreted by the cell and is an extension of the cell into the wider environment. The key traits involved are substrate elasticity, density, and configuration of attachment points; correct pore and fiber dimensions; and substrate composition. Fabrication of synthetic versions of any ECM type requires precise engineering at the microscale and even more precise engineering at the nanoscale because they provide many varied cues for the development of specialized tissues. The difficulty for the tissue engineer is that these features vary with time and in space[13]. Fabricating cell scaffolds with these features and properties is absolutely necessary to simulate an effective stem cell microenvironment.

Recreating the microenvironment with polymer biomaterials

The capability to sustain stem cells and stem cell built tissues, outside the body needs substantial improvement to attain clinical standards (Table 1). This is because, once removed and isolated from tissues stem cells rapidly lose their status, function and viability. The loss of proper intrinsic stem cell function happens because the support network of other cell contacts, contacts to matrices and the captured insoluble adhesion proteins and support cells are no longer present. Other influences in many culture systems deactivate stem cells such as, exposure to shearing forces[14].

Better strategies are really needed to capture stem cells, their progeny and the support cells inside privileged microenvironments, where they do not lose their unique intrinsic characteristics but, where their specialization can be programmed, maintained, and regulated for lifelong residence within the patient's own tissues[14,15]. Artificial life support systems dedicated to stem cells are being modeled on the structural design and composition of the ECM. An increasingly prolific strategy has been to use the ECM directly, removed of its cells[16]. Alternatively polymer copies are made of the ECM

Table 1. Current problems in keeping stem cells healthy and making them fit for transplantation

Problems in vitro	Problems following transplantation
Mechanical damage	High mortality
Loss of hard-wired properties	Do not migrate to damaged tissue
Differentiate into specialized cells	Do not integrate with host tissue
	Differentiate into specialized cells

structure. It is highly important that the structure and composition are prepared correctly. For this reason, basement membrane and other ECM components have been used to coat tissue culture surfaces in 2D to promote attachment and improve viability and growth of stem cells. Matrigel™ [17] and Geltrex™ [18] are actual ECM derived substrates used to control stem cell behavior and to propagate them within tissue culture systems. Matrigel is a proteinaceous hydrogel derived from sarcoma cell oversecretions that closely resembles the composition of native ECMs in many different tissues. Geltrex is a soluble basement membrane extract containing the primary factors of the ECM such as, laminin, heparan sulphate, and collagens. To have more regulation and control over cell responses it is necessary to be able to build tailor-made ECMs using a combination of natural molecules, purely synthetic molecules, and mixtures of the two. The most promising substrates that increasingly match native ECMs are protein polymers and synthetic polymers with oligopeptide additions; adhesion receptors; soluble and insoluble ligands that increase cell interactions; and stimulate natural tissue re-modelling[19,20]. The best examples of nanofibrillar scaffolds are those that develop in physiological conditions and incorporate cells. The drawbacks with all these material options are that they are quite intricate and complicated, they are often made in conditions intolerable to cells, and they do not proficiently accommodate cells during synthesis. Cell coating is a man-made microenvironment alternative where the materials chemistry is carried out simply, in physiological solutions, and is built around and incorporates cells and groups of cells.

Individualized microenvironments using biopolymers

The field of cell encapsulation is promising and could be a simple and effective means of processing stem cells and promoting their intrinsic functions for medical therapeutic potential. To further the clinical utility of such coatings it has been necessary to reduce the volume of encapsulation to increase diffusion rates into encased cells and reduce the volume of implanted cell masses[21].

We review the development and potential of nanometric coatings and micrometric coatings for stem cell therapy. Using this approach, cells are safely and spontaneously incorporated inside hydrophilic hydrogel biomaterials with many of the properties of natural ECMs such as, viscoelasticity, diffusive transport, attached growth factor proteins, and nanofibril networks[4].

Cell encapsulation has played a significant role in treating diseases arising from loss of cell function such as, Alzheimers, liver failure and, where it has been experimentally demonstrated, diabetes[22-26], because it can be highly effective at replacing diseased and defective cells with fresh replacements while providing a protective, selectively permeable barrier against immunological cells (Fig. 1). Encapsulation environments are also effectively used to promote tissue regeneration and improved targeted delivery of drugs and genes. The purpose of reducing the encapsulation to a thin layer is to increase efficiency of the procedure and the effectiveness of targeting biological encapsulates.

There may be important advantages to have stem cell microenvironments that are arranged around individual cells related to biology and processing. The most important advantage is that the presence of smaller volumes of matter reduces the problem of limited diffusion of respiratory gases, ions, and nutrients typically incurred inside large volumes of hydrogel. This can be a problem for cell viability inside microcapsules. Other advantages are that environments can be precisely tailored to suit cell type and even cell phenotype, be used to select a specific cell type from unwanted cells in the same suspension and create conditions that more efficiently target the delivery of genes and growth factors concentrated at the cell surface. Finally, coating could facilitate extended aggregations of cells, possibly into self-organized hierarchies, by designing coatings with cell recognition and adhesion molecules decorating the outer surface. There has been increasing interest in coating clinically relevant cells inside thin layers to reduce the overall volume of transplanted cells and increase diffusion[23]. Other studies have developed ultrathin coatings to establish cell survival in 3D cultures and to enhance

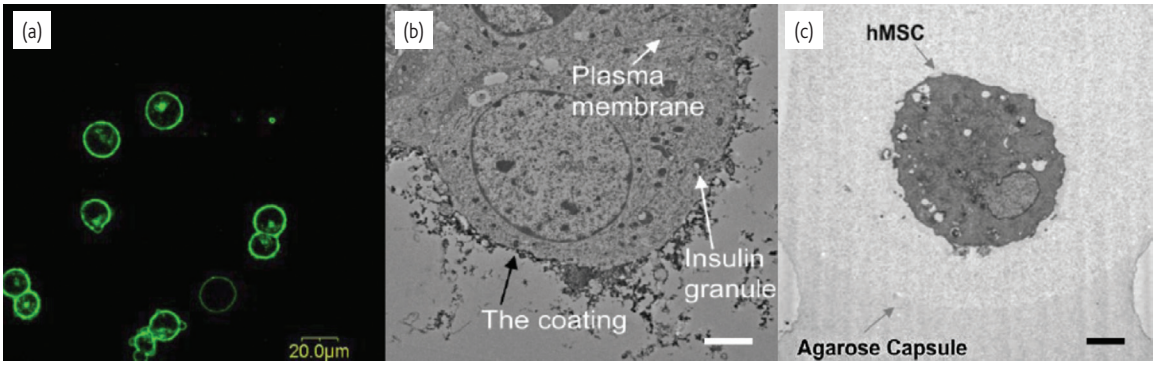

Fig. 1 Typical examples of individual cell and cell aggregated nanometric and micrometric coatings with selected biomaterials. (a) A confocal fluorescence microscope image of HEK293 cells coated with PEG-phospholipid layer attached to the cell membrane. The layer of biomaterial is combined with a green dye. (b) A TEM image of a pseudoislet following coating with five layers of chitosan, alginate, and chitosan/PC chondroitin-4-sulfate natural-origin biomaterials with a thickness of approximately 100 nm (scale bar= 2 μm). (c) TEM image of a human mesenchymal stem cell (hMSC) embedded inside its own agarose ultra thick "bulk" coating forming a small capsule approximately 60 μm in diameter (scale bar= 5 μm). Figures (a) and (c) reproduced with permission from Elsevier[22,42]. Figure (b) Reprinted with permission from[24]. © 2010 American Chemical Society.

Table 2 Microcoating and nanocoating methods for pancreatic islet cells and mesenchymal adult stem cells

Insulin producing pancreatic islet cells					
Clinical use	Cell origin	Coating method	Coating thickness	Coating substrates	Ref.
Islets of langerhans	Hamster derived pancreatic islets	Cell membrane anchoring	3 – 5 mm	Amino-terminated PEG-phospholipids Sodium alginate and Poly-L-Lysine	22
Pancreatic islet transplantation	Mouse pancreatic Insulinoma b- cell line	Layer-by-Layer (LBL) Nanofilms	100 nm	Chitosan/ alginate Phosphorycholine-chondroitin-4-sulfate	24
	Human pancreatic islet cells	Layer-by-Layer (LBL)	10 – 20 nm	PEG-phospholipid PAH-PDADMAC-PSS*	27
Transplantation device and Immune protection	Human kidney cell line Human liver carcinoma cell line	Emulsification	10 – 30 μm	Sodium alginate/ calcium alginate and PLO*	32
	Rat derived pancreatic islets	Chemical grafting	7 – 20 μm	Monomethoxy-PEG*	33
	Human kidney cell line Human liver carcinoma cell line	Gradient density centrifugation (conformal coating)	10 – 25 μm	HEMA-MMA*	34
Islets of langerhans	Pig derived pancreatic islet cells	Interfacial photo-polymersiation	40 – 80 μm	PEG diacrylates*	35
Mesenchymal adult stem cells					
Clinical use	Cell origin	Coating method	Coating thickness	Coating substrates	Ref.
Stem cell therapy and stem cell tissue regeneration	Mouse MSC	Layer-by-Layer (LBL)	6 – 9 nm	Poly-L-lysine Hyaluronic acid	28
Stem cell therapy and stem cell tissue regeneration	Human bone marrow	Colloidal precipitation	100 nm	Calcium phosphate/ amino acids (arginine and asparagine)	29
Cell therapy: Human marrow stromal cells delivery and apoptosis prevention	Human bone marrow stromal cells	In situ gelation	60 μm	Agarose	42
Stem cell therapy and stem cell tissue regeneration	Human bone marrow	Antibody connectors	3 – 5 μm	Chitosan/ CaP sodium alginate	
Stem cell therapy and stem cell tissue regeneration	Human bone marrow	Antibody/ adhesion peptide connectors	3 – 5 μm	Chitosan/ Sodium alginate	

*PEG- Polyethyleneglycol; CaP-Calcium phosphate; PAH-Poly-(allylamine hydrochloride)), PDADMAC (poly-(diallyldimethylammonium chloride)), PSS-(poly-(stryenesulfonate); PLO-Poly-L-Ornithine.

regulation and control of specialization for cell therapy and tissue regeneration. We review the current range of formulated cell coatings designed for cell therapy which differ in thicknesses, biomaterials, and their modes of attachment (Table 2).

Cell coatings with polymer biomaterials can be either deposited at the surface of the cell membrane, secured by intramolecular forces, or anchored[22] into the membrane, with thicknesses on the microscale (5 – 550 μm) and the nanoscale (6 – 100 nm) depending on the method used for application and attachment (Table 2)[23-25]. Primarily, the target cells for coating encapsulation in cell therapy have been pancreatic islets[23-27] but now there are new opportunities to enhance the therapeutic potential of adult mesenchymal stem cells with this technology. We start with the important and significant findings from nanometric coatings followed by micrometric coatings.

Cell nanocoatings using polymer biomaterials

Nanocoatings have provided a low impact protective packaging in pancreatic islet cell replacement therapy for the potential treatment of type 1 diabetes[26]. So for example, nanoscale coating of the pancreatic islets has been performed with consecutive layers of synthetic polymers: PAH, PDADMAC, and PSS[27]. Repeated deposition of nanothin films of material around groups of cells was made possible by the presence of electrostatic attractive forces at the cell membrane surface that attracted the oppositely charged substrate. Insulin producing pancreatic beta cell pseudoislets have been coated in consecutive layers of the marine derived biopolymers, chitosan, and alginate with nanoscale thicknesses to confer immunoprotection and reduce interference with cell metabolism[24]. The spheroid morphology characteristics of these cells were maintained and the metabolic activity was sustained by viable cells. The coating was attached to cell colonies through charge attraction alone, between cationic chitosan and the anionic cell membrane (Fig. 1)[24]. The principles and successful application of cell surface coatings on pancreatic cells has justified their use with mesenchymal stem cells, as there is a great need for technologies that can enhance their protection and anchorage and which support their activities in artificial culture.

Accordingly, mesenchymal stem cells have been coated in nanometric layers of *natural-origin* polymers and minerals (Table 2). Using the same sort of layer-by-layer method as for pancreatic islets, researchers have coated mouse derived mesenchymal stem cells inside five layers of hyaluronic acid and poly-L-lysine substrates 6 – 9 nm thick to provide a suitable environment for the differentiation and promotion of cell activities (Table 2)[28]. More significantly, coatings may be used to specialize human MSC to produce bone tissue. Gonzalez *et al.* coated primary human mesenchymal stem cells within a nanometric layer of calcium phosphate, functionalized with amino acids, to generate an immediate mineralized environment that promoted bone formation[29] (Fig. 2). The coating process is almost instantaneous and has an efficiency approaching 100 %. A supersaturated colloidal solution of calcium phosphate nanocrystals, combined with an amino acid to modulate crystal shape and size, measuring less than 100 nm is mixed with cells in suspension and at the membrane surface they become less soluble leading to deposition at the solid cell surface[29].

Coatings are not only applicable to cells in three-dimensional suspensions, but also to cells growing in flat monolayers in two-dimensional cultures. On closer examination using transmission electron microscopy it was found that the nanorods were extensively internalized into the cytoplasm explaining why the MSCs were turning into bone-like cells and why the presence of nanorods can establish a potential cargo route for genes and proteins.

This fact led to experiments to determine whether genes could be carried into the cell via these calcium phosphate particles. It is well known that calcium phosphate is a good transfection agent because of its strong complimentary electrical charge to deoxyribose nucleic acid (DNA) molecules. The result was successful transient transfection of the coated primary cells which are much more alike to their native counterparts than a transformed cell line (Fig. 3). Continuation studies showed that the coating was an effective substitute for osteogenic supplements in culture media and could capture endogenous growth factor proteins in the coating[30,31].

Cell microcoatings using polymer biomaterials

Many of the methods for coating deposition with nanometric resolution onto the cell surface are used to generate micrometric coatings[32-35].

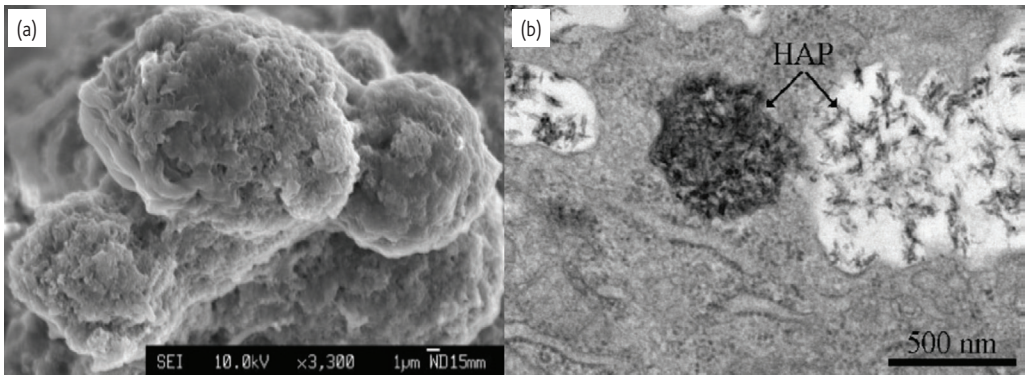

Fig. 2 Calcium phosphate nanocoating of hMSC to promote differentiation into bone cells. (a) Scanning electron microscope (SEM) image of a mesenchymal stem cell aggregate that has been coated with arginine-functionalised hydroxylapatite nanoparticles and nanorods. (b) Transmission electron microscope (TEM) image of hydroxylapatite nanorods surrounding the mesenchymal stem cell (MSC) membrane (right). Reproduced from[29] with permission from Wiley Publishers Ltd, © 2007.

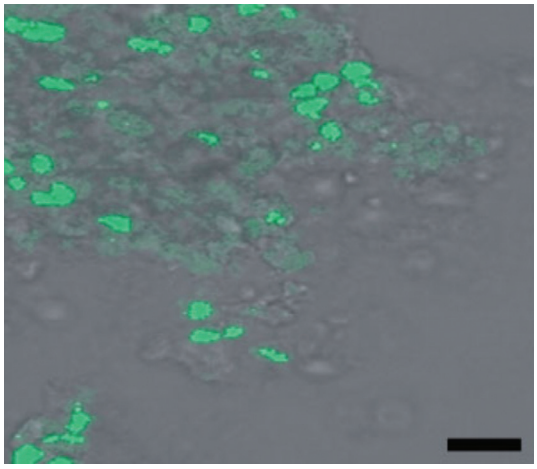

Fig. 3 Calcium phosphate nanocoatings can be used to help transfer genes into individual MSCs. Microscope image showing green fluorescent protein (GFP) emission by a 3D pellet of human mesenchymal stem cells pre-coated with hydroxyapatite particulate nanocoatings (grey speckling) carrying bacterial plasmid expression vectors with a GFP gene insert (scale bar= 50 μm). Reproduced from[29] with permission from Wiley Publishers Ltd, © 2007.

So far the majority of coatings employ continuous cell lines to model the potential for therapeutic use, and there is only one example where coating has been added to pancreatic islet cells and shown efficacy[35].

In contrast to cell nanocoatings, micron thickness cell coatings, ranging between 7 – 80 m, provide larger volumes of packing space for important proteins and genes that regulate encapsulated cells in which elution and delivery can be sustained over longer times. The need for a blood supply to each cell sets a limit for coating thickness, however. Furthermore, consecutive layering of individual microcoatings can help to recreate gradations in soluble growth factors from the cell surface to the capsule surface, for example, known to exist in natural tissues. In a crucial step to enhance stem cell preservation in an encapsulated environment the supporting cells from the stem cell niche can be immobilized inside one or more of these layers around the individual stem cell. The preferred substrates for coating are crosslinked hydrophilic hydrogels because they best mimic natural tissue viscoelasticity, diffusion properties, and flow[4].

Microcoatings can be applied either to 2D adherent cells or directly to 3D rounded up cells in suspension. The coating of cells in a three-dimensional rounded up conformation may confer a number of functional advantages over cells that spread onto conventionally prepared scaffolds. Diffusion is much less limited because there is a considerably lower substrate volume surrounding each cell; and with much less bulk substrate, external forces which can play an important role in regulating cell responses, may not be as dissipated compared to inside of a large block of substrate. Coated cell populations can be readily injected in a carrier fluid. Overall this results in a much more efficient use of biomaterial substrates. Selective coating using targeted antibodies and lectins enables specific phenotypes to be captured from heterogeneous mixtures. This specification of binding to cell surface membrane proteins could enable new connections to form with other cells and exogenous matrices through antibodies decorating the coating surface.

The *in vitro* deposition of materials onto 3D rounded-up cells with micron scale thicknesses is possible by repeated layering of consecutive negatively and positively charged polyelectrolytes to increase coating stability[36]. The significance and utility of this mode of therapeutic microcoating is that it can be carried out in a minimum of two incubation and washing steps and is also less expensive and time consuming than current standard alternatives. It is a fact that most cells are not viable when they are suspended in media because they need solid surfaces to latch onto[37]. However, these coatings have been shown to provide a solid surface and an anchorage which maintains their viability. With all existing coatings the long-term viability and function of encapsulated cells remains uncertain.

Individualized polysaccharide coatings for MSCs

Coatings that include more biological functions that can be individualized to any cell type may enhance survival and function for cell therapy and tissue regeneration in the long term. To meet this challenge we have recently developed a nanometric and micrometric coating which has combined utility in cell selection, protection, control of specialization, transplantation, and targeting of important therapeutic biomolecules such as growth promoting proteins and genes. In pilot studies we have successfully used natural-origin polysaccharide substrates to coat human bone marrow derived progenitor cells in a three-dimensional suspension and on cell monolayers using antibody and peptide connectors between the cell membrane and biomaterial substrate (Fig. 4). Coating involves a small series of consecutive immersions in an antibody solution, biotin solution, and polysaccharide solution. This procedure has been successfully carried out on primary human mesenchymal stromal cells (Fig. 4) and on adult

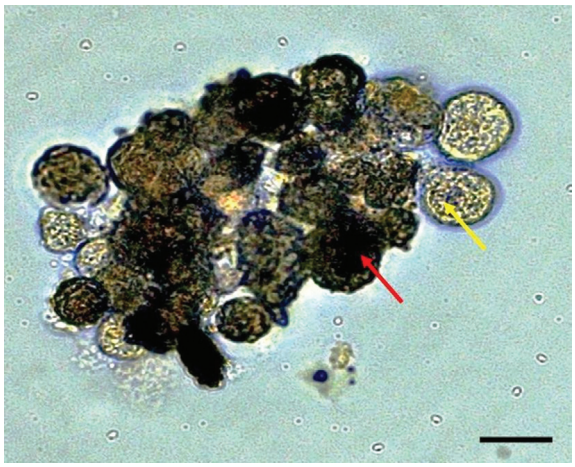

Fig. 4 An example of polysaccharide microcoating of human bone marrow derived progenitor cells using antibody connectors. A microscope image (bright field) of a small group of combined sugar coated and uncoated primary human mesenchymal stromal cells (derived from bone marrow) suspended in media, immediately after all coating steps have taken place. Coating was carried out using human alkaline phosphatise (ALP) antibody connectors bridged with avidin and biotin proteins. The red arrow indicates a coated cell, giving it a darkened appearance, while the yellow arrow points to an individual cell left uncoated because it has not secreted ALP (Scale bar= 10 μm).

primary limbal stem cells. Inside these localized sugar environments the human cells are healthy and stable. It has also been possible to coat small clusters of cells inside the sugar coating. These protected cells can break out of their temporary fabricated cocoon from a few days up to a week or more, as governed by the chemistry of the sugar coating. Carbohydrate (sugars, as glycans oligosaccharides and proteoglycans) are necessary to complete all the necessary functions of the ECM, as functional adjuvants to proteins and the stem cell niche and play a critical role in all aspects of stem cell biology[38]. This is important for maintaining cell viability and attachment. Research is being carried out where sugars are being added to the cell membrane and the glycocalyx (the cells natural sugar coating) to modulate metabolic pathways inside the cell[38]. It may also allow for the selection of any cell phenotype according to the functional proteins expressed on their membrane surface.

In addition, a new microenvironment connected onto the cell membrane with antibodies and lectins may be used as a potential model for studying the effect of specific extrinsic signals on stem cell regulation[39]. So, for example, when embryonic stem cells are cultured inside bioreactors and microfluidic devices they are subjected to mechanical forces which at certain strengths can destabilize gene function, protein expression and alter their phenotype[40]. Our specialized microcoating also functions as a device for drug and gene delivery which is designed to target specific cells and direct cell migration towards specific tissues and anatomical sites inside the body.

Outlook

Coating ultrathin layers of biomaterial around therapeutic stem cells is a fresh and uncomplicated method to temporarily deputize any natural ECM. These coatings help to maintain the unique intrinsic properties of stem cells outside the body so that they can be efficiently used to promote regeneration and protect for transplantation. Coated stem cells should be easier to manipulate and process as well as better protected, directed, and regulated than normal uncoated stem cells. Coatings are designed to be temporary substrates ideally made from crosslinked hydrophilic hydrogels because they best mimic natural tissue viscoelasticity, diffusion properties, and flow patterns. As with all present ECM analogues they lack the dynamism of a living matrix but the potential is there to influence cells in positive ways that facilitate stem cell bioprocessing, harness intrinsic stem cell properties and promote tissue regeneration, without needing retroviral transfection procedures. We also foresee that such coatings have the potential to protect stem cells against mechanical damage and ice crystal damage following cryopreservation by attempting to match the design of polysaccharide capsules with those evolved by extremophile bacteria to protect from extremes of temperature and pressure. According to Kotobuki *et al*. viability of cryo-preserved human mesenchymal stem cells was as high as 90 % and these mesenchymal adult stem cells possessed high differentiation potentials[41]. Most cells are not as robust and it is important to provide protective environments free of toxic and synthetic products. The promise of this type of technology reaches further as: recruiter of exogenous stem cells, biochemical re-modeling of stem cell surfaces, targeted cell nutrition modules, living drugs, and gene carriers. mt

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