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Discovery of a Novel Polymer Enabling Defined Human Pluripotent Stem Cell Expansion and Multi-Lineage Differentiation

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Human pluripotent stem cells (hPSCs) have been proposed for various regenerative medicine, tissue engineering and drug discovery applications due to their ability to self-renew and be differentiated into numerous lineages representative of the three embryonic germ layers[1-4]. However, for the potential of hPSCs to be realized, bioprocessing-scale culture systems are required that can manufacture clinically relevant numbers of cells in an economical and reproducible manner. For example, to replace damaged tissue after a heart attack, it has been estimated that more than 1 billion cells would be needed for each patient[5]. To facilitate the transition from research to industrial scale production of adherent cell types, the ideal culture system should comprise of both defined medium and substrate that can be readily-used with existing cultureware from which a *stem cell factory* can be constructed[12]. To meet this need, defined media formulations are available commercially for the expansion of hPSCs, including the widely-used StemPro[6] and mTeSR1[7]. These have improved reproducibility in hPSC culture by avoiding mouse embryonic fibroblast-conditioned medium (MEF-CM), the use of which remains commonplace and exhibits high batch variability[6-8]. Xenogenic components in the culture system create a barrier to clinical translation as they face greater regulatory hurdles. Nonetheless, there is still widespread use of the poorly-defined mouse sarcoma preparation, Matrigel™, as a cell attachment surface[9]. Therefore, the challenge is to find new materials from which to produce defined growth substrates for hPSCs that function with commercial defined media.

Polymeric materials show considerable promise as culture substrates due to their ease of manufacture from inexpensive, readily-available monomers and their industrial scalability[10]. High throughput screening methods have been used to accelerate the search for new polymers for hPSC culture since unbiased screening can be carried out with current knowledge of hPSC-substrate interactions[11-14]. Synthetic substrates identified using polymer microarrays have been shown to support the clonal growth of hESCs[15]. However, these materials require a preconditioning step with vitronectin, and scale up from the 300μm diameter micro array spots was not achieved. Despite these efforts, until now an effective polymeric growth substrate on which both hPSC expansion and subsequent differentiation can be induced has yet to be developed[10].

In this report, we have achieved hPSC expansion on a xeno-free polymer substrate in defined, commercially available culture media (StemPro and mTeSR1) and multi lineage differentiation on the same polymer. A range of candidate polymers were identified using a high throughput screening microarray methodology developed to sample an unprecedented chemical space (141 monomers, polymerized alone and mixed to form 909 unique polymers, tested in 4356 individual assays). This allowed us to identify a novel copolymer substrate that achieves pluripotent hPSC expansion without the need for protein preconditioning. A simple procedure has been developed to coat standard cultureware, exemplified for the commonly used 6-well plate format. The same polymer was shown to support hPSC differentiation into representatives of the three germ layers, namely cardiomyocytes, hepatocyte-like cells and neural progenitors.

A multi-generation high throughput polymer microarray screening methodology incorporating high throughput surface characterization (HT-SC)[16] was used to identify

materials that can support HUES7 (hESC) cell attachment and pluripotency in the widely used commercial defined, serum- and feeder-free medium, StemPro. The first-generation array, consisting of 141 monomers of wide chemical diversity, was printed using metal pins to transfer the liquid monomers onto poly(2-hydroxyethyl methacrylate) (polyHEMA) coated glass slides containing 6 replicates of each homopolymer (**Figure 1**a - monomer structures presented in Figure S1 and Table S1).[17] Polymer microarray spots of diameters ranging from 250-400 μm were formed by UV photopolymerization using a modification of methods described previously [18,19] Arrays were preconditioned for 1 hour in StemPro medium prior to seeding with $1x10^6$ HUES7 hESCs and culturing for 24 hours. Samples were fixed, stained for OCT4 expression (an indicator of pluripotency) and images acquired using an automated fluorescence microscope. Images were automatically processed to quantify cell response to each polymer spot (using CellProfiler software). This initial screen was used to identify 24 'hit' materials on the basis of their ability to support high HUES7 hESC attachment across six replicates, whilst maintaining OCT4 expression (>90%) (Figure 1b and Figure 1c – 'hit' monomer structures presented in Figure S3).

To explore the effect of copolymerization the 24 hit monomers were mixed pairwise in a combinatorial manner (70/30 v/v mixtures) to form a second-generation array design comprising 576 unique materials in triplicate (Figure 1d). Quantifying OCT4 positive HUES7 cell attachment after 24 hours on the second-generation array in the same way as before identified a refined list of nine monomers that displayed high hPSC attachment as homopolymers and copolymers across the array (up to 100 cells/per spot) (Figure 1e - 'hit' monomer structures presented in Figure S3). Synergistic combinations of monomer were noted providing greater hPSC attachment than their homopolymer constituents. A third-generation array was used to explore these hit monomers as copolymers at varied composition ratios to determine whether substrates could be improved further for HUES7 cell

attachment and maintenance of OCT4 expression during the first 24 hours of culture. The 9 lead monomers were mixed combinatorially, utilizing additional ratios (10, 20, 30 and 40% v/v) to produce a third-generation array of 297 materials (Figure 1f). To make the assay more stringent in order to identify the most robust candidate polymers, the cell seeding density was reduced. This led to a significant reduction in cell attachment across the array, with only 90 copolymers showing significant cell adhesion.

The best performing polymers in the third-generation array all contained monomer 5 (N-(4-Hydroxyphenyl)methacrylamide) (HPMA), which was able to support HUES7 adhesion both as a homopolymer and as a copolymer (up to 56 ± 7 cells/per spot) (Figure 1g). Inclusion of HPMA as a minor component (10 – 40% v/v) with monomers that performed poorly as homopolymers dramatically increased the performance of the resulting copolymers. For example, monomer 26 (Lauryl methacrylate – M26) supported no attachment of hPSCs across the array as a homopolymer. However, inclusion of HPMA as a minor (10% v/v) or major (90% v/v) component with M26 increased hPSC attachment (Figure 1g). This "hit", monomer supported the highest HUES7 cell attachment as a homopolymer within the third-generation array. The increase in cell attachment by addition of other monomers was determined to be too small to justify taking forward any of the copolymer formulations and polyHPMA was defined as the lead candidate to be taken forward for hPSC expansion studies.

High throughput surface characterization (HT-SC) was performed to determine the actual chemistry of the surface rather than assuming it to be the same as the bulk monomer composition. This has previously been shown to be important when copolymers are formed on slide, since surface segregation may result in unexpected surface chemistries and printing errors might lead to misidentification of hit materials[20,21]. Surface chemical analyses including time-of-flight secondary-ion mass spectrometry (ToF-SIMS), X-ray photoelectron

spectroscopy (XPS) and water contact angle (WCA) measurements were acquired from all polymers. No correlation between cell attachment and surface chemcial analysis could be found for the first- or second-generation arrays (Figure S4 and Figure S5). This is consistent with previous observations for stem cell-polymer interactions[15,21-23].

To investigate the excellent cell attachment performance of HPMA-containing copolymers in the third-generation array, the intensity of the ToF-SIMS ions characteristic to HPMA ($C_7H_4NO_2^-$) and M26 ($C_9H_{11}O_2^+$) were compared in the spectra of the homopolymers and copolymers (Figure S6). The intensity of the characteristic $C_7H_4NO_2^-$ secondary ion is highest in the polyHPMA homopolymer and decreased dramatically upon inclusion of M26. This can be explained by surface enrichment of M26, possibly in the monomer mixture prior to UV photopolymerization. The intensity of $C_9H_{11}O_2^+$ secondary ion characteristic of M26 is consistent with this explanation. To quantify the amount of HPMA at the surface these materials, XPS analysis was employed using the elemental composition using nitrogen as a marker for polyHPMA (Figure 1h). The relative amount of nitrogen in polyHPMA homopolymer ([N]=4at%) is reduced by half upon inclusion of 10% M26 (1.9%). The amount of nitrogen in the XPS spectra follows a similar trend to the $C_7H_4NO_2^-$ ion in the ToF-SIMS spectra for these materials confirming that M26 is enriched at the surface. Thus, despite there only being relatively low levels of polyHPMA at the surface of all copolymers than the uniform distribution expected of statistical copolymers, such small amounts are all that is required to encourage cell attachment, illustrated schematically for poly(HPMA-co-P26) (90:10% v/v) in the third-generation microarray (Figure 1i).

Focussing on the lead monomer, HPMA, scaled up materials of polyHPMA in 6-well plates were manufactured (see methods) and analyzed by ToF-SIMS to determine if the surface chemistry was consistent with polyHPMA in microarray spots. ToF-SIMS peaks characteristic of HPMA were observed at *m/z* = 108 and 109 ($C_6H_6NO^+$ and $C_6H_7O^+$

168 respectively) from both microarray spots and from coatings scaled up to coat 6-well plates
169 (Figure 1j). In the third-generation polymer microarray, we observed additional peaks at *m/z*
170 = 45 and 113 ($C_2H_5O^+$ and $C_6H_9O_2^+$ respectively), which are characteristic of polyHEMA
171 (Figure 2c). This indicated that the underlying polyHEMA support material had intermixed
172 with the deposited monomers to be present at the surface of these spots of this array. Given
173 the high level of hPSC attachment on the polyHPMA spots in the third-generation polymer
174 microarray (Figure 1g), we reasoned that polyHEMA might be a beneficial additive and
175 explored this as a co-monomer in scale up.

176 For wide applicability, a synthetic culture substrate needs to be optically transparent, cost-
177 effective, compatible with common TCPS cultureware and be scaled up to coat cultureware
178 that could support the expansion of cells at an industrial scale. Previously, promising
179 materials for hPSC culture identified on polymer microarrays have been restricted to use as
180 arrayed spots or small growth areas such as coated coverslips or slides incompatible with
181 scalable cell manufacture[12,13,15]. To demonstrate scale up from the microarray spots we
182 developed a method to coat commonly used polystyrene cultureware with HPMA.

183 Scale up to well plates of polyHPMA using *in situ* UV polymerization methods were
184 unsuccessful because of difficulty removing unpolymerised residual monomer (as detected by
185 ToF-SIMS) from the thick polymer coatings, which caused cell death (Figure S7 and
186 Supplementary Methods). We therefore employed a prepolymerization approach where
187 solution of polyHPMA was presynthesized and subsequently coated onto plasma etched
188 tissue culture polystyrene (PE-TCPS) cultureware (see methods and Figure S8). Using a
189 prepolymerized solution to coat cultureware is convenient as large quantities of polymer can
190 be synthesized in one batch that can be applied in a simple coating procedure, which can be
191 performed routinely by hand or by industrial automation using existing TCPS manufacturing
192 procedures that involves plasma activation[24]. The substrate formed after evaporation of an

ethanolic polyHPMA solution was transparent, however cracking was observed after solvent evaporation that developed on storage in cell culture incubators (Figure S9). Given that low levels of HEMA were detected in the polyHPMA micro arrays (Figure 1k) we chose this monomer to copolymerize with HPMA to prevent cracking.

The poly(HPMA-co-HEMA) copolymer was synthesized and characterized in the same manner as polyHPMA (Figure S10 and Supplementary methods). Coatings consisting of poly(HPMA-co-HEMA) gave transparent wells which did not exhibit cracks after extended immersion in culture media (1 month) (Figure S11). ToF-SIMS analysis confirmed that HPMA and HEMA moieties were present at the surface of these materials (Figure 2d). To provide a quantitative elemental and functional characterization of the surface chemistry, XPS analysis was performed (Figure 2e and Figure S12). XPS analysis of polyHPMA from the array format revealed a lower elemental nitrogen content ([N] = 4.0 at%) compared to scaled up polyHPMA and poly(HPMA-co-HEMA) (8.7% and 7.7% respectively). These measurements indicate the presence of the polyHEMA from the underlying substrate diffusing to the surface of the arrayed material during the printing process and supports the ToF-SIMS analysis that discovered increased HEMA moieties and decreased HPMA moieties compared to the spectra of polyHPMA and poly(HPMA-co-HEMA). The prepolymerized material can also be seen to have approximately 10 mol% HEMA at the surface. The high quality transparent coatings and presence of HPMA and HEMA chemical moieties at the surface meant that poly(HPMA-co-HEMA)-coated cultureware provided a suitable substrate to evaluate hPSC expansion.

Adopting the conditions identified above to fabricate poly(HPMA-co-HEMA) coated 6-well plates permitted attachment of hPSCs and their proliferation to confluence. We next sought to evaluate maintenance of pluripotency to determine whether hPSCs could conform to accepted criteria, including serial propagation for at least 5 passages whilst retaining karyotype

stability and expression of pluripotency markers, as well as differentiation to representatives of the three embryonic germ layers (mesoderm, endoderm and ectoderm). We evaluated whether substrate preconditioning with culture medium or ECM proteins was a necessary step. Comparison of hPSCs seeded with or without preconditioning showed similar levels of attachment and distribution after 24 hours in StemPro. Similarly, medium exchanges led to expansion to confluency by 72 hours in both conditions (**Figure 2**a). Therefore, preconditioning was omitted during subsequent passages.

Propagation through 5 serial passages with accutase on the poly(HPMA-co-HEMA) substrate showed a consistent population doubling index of 81.3 +/- 8.5 hours (Figure 2b), retention of 46X,Y karyotype by G-banding of 30 cells (Figure S13) and expression of OCT4, TRA181 and SSEA4 in >93% cells, as measured by quantitative immunofluorescence using an automated plate reader (Operetta®) and high-content image analysis software (CellProfiler) (Figure 2c, Figure 2d and Figure 2e).

We sought to test whether the poly(HPMA-co-HEMA) substrate could support pluripotent expansion of hESC and hiPSC lines in different commercial culture media. Thus, cultures of the HUES7 hESC line and BT1 hiPSC line were initiated in StemPro and another commonly-used defined medium, mTeSR1[7]. In each case, consistent population doubling times were observed through 5 serial passages in the absence of any preconditioning step (Figure 2b). There was also retention of stable karyotype (46,XY for HUES7; 46,XX for BT1) (Figure S13), and pluripotent markers of OCT4, TRA181 and SSEA4 by immunofluorescence (all >88%) (Figure 2c and Figure 2d). Poly(HPMA-co-HEMA)-coated cultureware can be stored for at least 6 months at ambient conditions and can be used off-the-shelf in the same way as general TCPS cultureware (Figure S14).

241 Since coupling hPSC expansion with differentiation would increase the utility of an
242 expansion substrate, we sought to evaluate whether the formation of representatives of each
243 of the three germ layers during human development could be induced by directing
244 differentiation on poly(HPMA-co-HEMA).

245 We directed formation of cardiomyocytes (mesoderm) by culturing two-dimensional
246 monolayers of hPSCs on poly(HPMA-co-HEMA) with modulators of TGF-b superfamily
247 (activin A and BMP4)[25] and WNT (KY02111[26] and XAV393[27]) pathways. In the same
248 time course as hPSCs differentiated on Matrigel (12 days), beating clusters of
249 cardiomyocytes formed (see Supplementary Video 1), which were shown by immunostaining
250 to be positive for α-actinin and cardiac troponin-T staining (**Figure 3**a and Figure 3b).
251 Functional analysis of the differentiated cells by patch clamp showed they had
252 electrophysiological characteristics similar to those previously published for hPSC-
253 cardiomyocytes[28], including a mean action potential duration (APD) of 417+102 ms (Figure
254 4c). Based on 90% / 50% repolarization values (APD90/APD50), these cultures contained
255 ventricular (APD90/APD50 of ≤ 1.3), atrial (≥ 1.8) and pacemaker (1.4-1.7) cardiomyocyte
256 subtypes[29] (Figure 3d).

257 Directed hepatocyte differentiation (endoderm) was achieved via an 18 day protocol[30] using
258 activin-A, Wnt3a, FGF, HGF and oncostatin-M to modulate signaling cascades.
259 Differentiated cell cultures on poly(HPMA-co-HEMA)-coated substrate expressed albumin,
260 AFP, HNF4A and A1AT and secreted AFP with comparable efficiency to Matrigel-coated
261 controls (Figure 3e, Figure 3f and Figure 3g).

262 Finally, we induced hPSC differentiation to neural progenitors, which arise from the
263 ectoderm germ layer. Dual SMAD-inhibition[31,32] with dorsomorphin and SB431542 for
264 seven days induced the formation of neural rosette-like colonies on poly(HPMA-co-HEMA)

substrates (Figure 3h, Figure 3i and Figure 3j). Neural progenitors produced on Matrigel and poly(HPMA-co-HEMA) displayed similar levels of PAX6 and SOX1 markers (PAX6: 78% ± 4% and 74% ± 8%; SOX1: 68% ± 11% and 69% ± 18% respectively).

In order to determine a mechanism for the hPSC adhesion to poly(HPMA-co-HEMA), antibody blocking assays were performed for key hPSC integrins. Blocking of the integrins β_1 and $\alpha_V\beta_5$ resulted in a significant reduction (>30%) in hPSC attachment to poly(HPMA-co-HEMA) when cultured in StemPro media (**Figure 4**a, Figure 4b and Figure 4c). Although hPSCs have been shown to express numerous integrins, including those of the α_1, α_2, α_3, α_5, α_6, α_7, α_V and α_{11}, and β_1, β_2, β_3 and β_5 families[9,33-36], only α_2, α_5, α_6, α_V and β_1 integrins have been shown to play a significant role in hPSCs adhesion to Matrigel coated culture surfaces[37,38], and only α_V integrins in hPSC adhesion to polymer culture surfaces without matrix coatings[15]. This is therefore the first report demonstrating a role for β_1 as well as α_V integrins in hPSCs adhesion to polymer culture surfaces without matrix coatings. Although individually $\alpha_V\beta_5$ binds vitronectin sites and β_1 binds fibronectin and laminin sites[37], it is likely that these two integrins interact in a complex manner to promote hPSC adhesion to sites present in the poly(HPMA-co-HEMA) chemistry or to proteins (most likely albumin) adsorbed from the medium (Figure 4d).

In summary, we have used a high throughput combinatorial approach to identify and develop a defined, synthetic polymeric substrate that supports hPSC pluripotency and expansion through serial passage in commercial defined media without the need for protein pre-adsorption. This was achieved for both hESCs and hiPSCs. Additionally, directed differentiation was achieved on the hit polymer, poly(HPMA-co-HEMA), to representatives of each of the three germ layers, including cardiomyocytes (mesoderm), hepatocyte-like cells (endoderm) and neuro-ectoderm (ectoderm). Thus, poly(HPMA-co-HEMA) fulfills all the current culture requirements for the clinical use of stem cells within regenerative medicine

and can be scaled up by coating onto cultureware to be used *off-the-shelf*, providing a cost-effective alternative to commercially available hPSC expansion substrates. The expansion of hPSCs and production of terminally differentiated cell types without the influence of undefined and xenogenic matrix protein coatings provides a robust platform for the industrial scale production of hPSCs for regenerative medicine applications and therapies.

Experimental

Preparation of polymers: polyHPMA and poly(HPMA-co-HEMA) were prepared via a thermally initiated free radical polymerization in an ethanolic solution with the addition of 2,2'-azobis(2-methylpropionitrile) (AIBN – 1% w/w to HPMA). The isolated and dried polymers were dissolved in ethanol (5% w/v) and added into TCPS 6-well to cover the base of each well plate directly after oxygen plasma activation. The solvent was allowed to evaporate under ambient conditions for 24 hours prior to hPSC culture. Complete detailed methodolody of polymer synthesis, characterization and all cell culture protocols can be found in the supporting information.

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Figure 1. Multi-generation microarray screening strategy and high throughput surface characterization. (a) A first-generation array of wide chemically diversity consisting of 141 monomers was used to screen for hPSC attachment. (b) Materials were ranked by hPSC attachment (six replicates) after 24 hours in culture whereupon cells were quantified by DAPI and OCT-4 staining. (c) Phase (left) and fluorescence (right) images of an example 'hit' material from the first-generation. Scale bar = 250 μm. Fluorescence image is a combined image of both DAPI (ex = 410 nm, em = 470 nm) and OCT4 (ex = 530 nm, em = 630 nm). (d) 24 'hit' materials were mixed pairwise in a combinatorial manner to produce a second-generation of 576 unique materials. (e) These materials were assessed and ranked in a similar way to the first-generation array. (f) A third-generation array was printed from 9 common monomers that formed the hit copolymers in the second-generation array but were mixed in further ratios to form an array of 297 materials. (g) Materials were ranked to identify lead compositions for scale up. (h) XPS analysis of polyHPMA, P26 and copolymers thereof to determine the actual surface chemistry. Line is drawn to guide the eye. (i) Proposed surface chemistry of poly(HPMA-co-P26) (90:10% v/v) identified from ToF-SIMS and XPS analysis of third-generation microarray. (j) ToF-SIMS and (k) XPS analysis of polyHPMA in the

436 third-generation array revealed polyHEMA at the surface which was incorporated as a co-
437 monomer for scaled up polymers to assess hPSC expansion.

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439
440 **Figure 2.** hPSC expansion through serial passage. (a) Phase images showing that hESCs and
441 hiPSCs were able to attach to poly(HPMA-co-HEMA) substrates at 24h in and expand to
442 confluence and compaction at 72h. (b) Growth curves for hESCs and hiPSCs showing
443 consistent cell population doubling (CPD) times on (□) poly(HPMA-co-HEMA) versus (O)
444 Matrigel controls for 5 passages for varied media conditions. (c) Positive
445 immunofluorescence for pluripotent markers OCT4, TRA181 and SSEA4 following serial
446 passaging of hPSCs on poly(HPMA-co-HEMA). Scale bar = 250 μm. (d) hPSCs on
447 poly(HPMA-co-HEMA) maintain comparable pluripotent marker expression levels versus
448 Matrigel controls, with OCT4, TRA181 and SSEA4 expression >88% (e) Example of
449 automated high content image analysis displaying DAPI (white line) and OCT4 (yellow line)
450 positive HUES7 cells on poly(HPMA-co-HEMA) in mTeSR1 medium. Scale bars = 100 μm
451 (left image) and 10 μm (right image).

452

Figure 3. Three germ layer directed differentiation of hPSCs on polymeric substrate. (a) Mesoderm differentiation on poly(HPMA-co-HEMA) induced positive α-actinin and cardiac troponin-T expression. Scale bar = 50 μm (b) Quantification of positive cardiac marker expression on poly(HPMA-co-HEMA) and matrigel displayed similar levels. (c) Electrophysiology of the spontaneously beating cardiomyocytes on poly(HPMA-co-HEMA) showed ventricular (APD90/APD50 of ≤ 1.3), atrial (≥ 1.8) and pacemaker (1.4 - 1.7) subtypes of cardiomyocytes, (d) with a mean action potential duration (APD) of 417 ± 102 ms (e) Endoderm differentiation on poly(HPMA-co-HEMA) induced hepatic marker expression (Scale bar = 100 μm) (f) in hepatocyte-like cells with active AFP secretion (g) Quantification of positive hepatocyte marker expression on poly(HPMA-co-HEMA) and matrigel displayed similar levels. (h) Ectoderm differentiation on poly(HPMA-co-HEMA) induced neurogenesis marker expression (Scale bar = 100 μm) (i) Quantification of positive neuroectoderm marker expression on poly(HPMA-co-HEMA) and Matrigel displayed similar levels. (j) PAX6 positive neural rosette formation on poly(HPMA-co-HEMA) following ectoderm differentiation (Scale bar = 1 mm).

Figure 4. Integrin blocking of hESCs on poly(HPMA-co-HEMA). hESCs adhered to poly(HPMA-co-HEMA) actively express the integrins (a) β_1 and (b) $\alpha_V\beta_5$. Scale bar = 50 μm (c) Following the blocking of the integrins β_1 and $\alpha_V\beta_5$ there is a significant reduction in hPSC adhesion to poly(HPMA-co-HEMA) in StemPro media. (d) Schematic of proposed hPSC adhesion mechanism through integrin binding to adsorbed media proteins on poly(HPMA-co-HEMA).

The table of contents entry should be 50−60 words long, and the first phrase should be bold. The entry should be written in the present tense and impersonal style.

A scalable and cost-effective synthetic polymer substrate that supports robust expansion and subsequent multi-lineage differentiation of hPSCs with defined commercial media is presented. This substrate can be applied to common cultureware and used *off-the-shelf* after long-term storage under ambient conditions. β_1 and $\alpha_V\beta_1$ integrin interactions with proteins adsorbed from media to the surface is critical in achieving cell attachment to this polymer.

Keyword

*A. D. Celiz, *J. G. W. Smith, A. K. Patel, A. L. Hook, D. Rajamohan, V. T. George, M. J. Patel, V. C. Epa, T. Singh, R. Langer, D. G. Anderson, N. D. Allen, D. C. Hay, D. A. Winkler, D. A. Barrett, M. C. Davies, L. E. Young, ★C. Denning, ★M. R. Alexander

Discovery of a Novel Polymer Enabling Defined Human Pluripotent Stem Cell Expansion and Multi-Lineage Differentiation

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