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Research Article

Immobilized hematopoietic growth factors onto magnetic particles offer a scalable strategy for cell therapy manufacturing in suspension cultures

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Hematopoietic therapies require high cell dosages and precise phenotype control for clinical success; scalable manufacturing processes therefore need to be economic and controllable, in particular with respect to culture medium and growth factor (GF) strategy. The aim of this work was to demonstrate the biological function, and integration within scalable systems, of a highly controllable immobilized growth factor (iGF) approach. GFs were biotinylated and attached to streptavidin coated magnetic particles. GF concentration during biotinylation, GF-biotin ratio, and GF lysine content were shown to control iGF surface concentration and enable predictable co-presentation of multiple GF on a single bead. Function was demonstrated for immobilized GMCSF, SCF, TPO and IL-3 in GF dependent cell lines TF-1 and M-07e. Immobilized GMCSF (iGMCSF) was analyzed to show sustained activity over eight days of culture, a two to three order of magnitude potency increase relative to soluble factor, and retained functionality under agitation in a microscale stirred tank bioreactor. Further, short exposure to iGMCSF demonstrated prolonged growth response relative to soluble factor. This immobilization approach has the potential to reduce the manufacturing costs of scaled cell therapy products by reducing GF quantities and offers important process control opportunities through separation of GF treatments from the bulk media.

Keywords: Growth factors · Hematopoietic · Immobilized · Manufacturing · Scalable

1 Introduction

The processing, or manufacture, of hematopoietic stem cells (HSCs) for therapeutic application has made significant progress in recent years. In the field of HSC transplant successful strategies have been developed for the use of expanded cell populations in support of an unexpanded unit to reduce time for myeloid recovery [1]. Emerging research suggests a single expanded cell population may soon be a viable treatment option [2]. Success is also being achieved with genetically modified hematopoietic cells for treating rare genetic abnormalities such as Wiskott-Aldrich syndrome or Adenosine deaminase deficiency [3–5]. Early stage research promises manufacture of mature lineage cells such as erythrocytes or platelets from progenitors. Wider adoption of such therapies will require scalable and controllable processes within the constraints of economic delivery.

A common factor in the manufacture of therapeutic cells is the reliance on soluble signaling factors, such as cytokines, to control growth and differentiation outcomes; current cell culture protocols primarily consist of defined cocktails of soluble factors. However, the use of soluble factors has limitations including rapid decay and homogenous dispersion in culture [6]. This prevents presentation of high concentrations of factor at the receptor relative to bulk distribution (reducing effective potency),

Abbreviations: ECM, extracellular matrix; GF, growth factor; GMCSF, granulocyte macrophage colony-stimulating factor; HSC, hematopoietic stem cell; iGF, immobilized growth factor; IL-3, interleukin 3; PEG, polyethylene glycol; PDs, population doublings; SCF, stem cell factor; SD, standard deviation; TPO, thrombopoietin

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and presentation of several discrete combinations of factors simultaneously to generate multiple microenvironments within a bulk culture. It also prevents recycling of active GFs independently from other factors in the milieu [7, 8]. Collectively these drawbacks reduce opportunities to decrease costs, of which media volume and supplemented factors form a substantial part, and hinder the development of more refined GF strategies to exert control over proliferation, commitment and heterogeneity of cell populations.

Surface adhered GFs are an alternative culture strategy to soluble. In vivo, a number of GFs (e.g. SCF and Flt-3L) can exist in both soluble and matrix (or membrane) bound forms [9, 10]. Matrix bound GFs associate with glycosaminoglycans (heparin or heparan sulfate) or proteins of the extracellular matrix (ECM) including fibronectin and collagen [7, 11]. Immobilization of GFs in vitro allows high localized concentrations of factor combinations which can induce synergetic signaling through mechanisms such as ligand multivalency and receptor clustering; this has been suggested to lead to distinct signaling pathways in comparison to their soluble counterparts [12]. Further, proteins such as SCF and Notch-1 ligand have distinct functional effects depending on their soluble or bound state [13–17]. Other functional attributes reported for iGFs include increased potency, prolonged response, and protection from proteolytic degradation and receptor-mediated endocytosis [18–21]. Immobilization of proteins within a culture system therefore has the potential to offer more control opportunities than soluble factors alone.

Achieving controlled cell fate outcomes from iGF is dependent on a suitably well mixed system, an appropriate mechanism of immobilization, and that the cell/iGF interaction will predictably scale. Soluble growth factors (sGF) will diffuse down a concentration gradient in a non-mixed or poorly mixed system; conversely iGF have a fixed locus leading to potentially heterogeneous exposure of individual cells. To address these criteria we have developed an immobilization strategy that is amenable to incorporation in a stirred tank bioreactor format. Immobilization of GFs in a culture process could be achieved via a wide range of methods and these are described comprehensively elsewhere [22]. The immobilization method selected was based on a Streptavidin coated surface treated with a biotinylated version of the GF of interest with a PEG linker between the biotin and the GF. This system has a number of attractive attributes including: click chemistry under benign reaction conditions, reagents compliant with clinical use, and the potential to tune steric presentation of GFs. To achieve scalability we immobilized onto magnetic particles that could be distributed at a controlled surface to volume ratio in a standard stirred tank bioreactor format. Such a culture format has extensive literature supporting control of culture conditions and scalability [23–24]. Further the magnetic properties enabled temporal control of GF exposure independently of the bulk medium. This approach enabled us to demonstrate functionality and selective temporal control of key clinically relevant hematopoietic factors GMCSF, SCF and TPO in a scalable production system.

2 Materials and methods

2.1 Growth factor immobilization

The immobilization method involves a two-step reaction:

2.1.1 Step 1 – Growth factor biotinylation

1.1. Lyophilized carrier free GFs (R&D Systems, UK) were re-suspended in PBS at a concentration of 50 µg/mL.

1.2. NHS-PEG_{2000}-biotin (Nanocs Inc., USA) was suspended in anhydrous DMSO at the required molar concentration for the individual experiment being carried out and used immediately.

1.3. Equal volumes of GF and NHS-PEG_{2000}-biotin were mixed in LoBind Protein Eppendorf tubes and vortexed for 2 h at room temperature.

2.1.2 Step 2 - Immobilization of biotinylated growth factors

2.1.2.1 2.8 µm streptavidin coated magnetic particles (Solulink, USA) were placed on a magnetic stand (MagnaRack™ Magnetic Separation Rack, ThermoFisher Scientific) and the supernatant removed.

2.1.2.2 Biotinylated GF solution from step 1.3 was added once with PBS and re-suspended in 100–500 µL of PBS cytometry stain buffer containing the required concentration of conjugated monoclonal antibodies (R&D Systems, UK) for 1 h at room temperature. Where conjugated antibodies were unavailable, antibodies were custom conjugated using an APC-Antibody conjugation kit (Solulink, USA). In parallel, iGF particles were also stained with an antibody isotype control. iGF particles were then washed once with PBS and re-suspended in 100–500 µL of PBS.

2.2 Quantification assays

2.2.1 Immobilized growth factor concentration

2 x 10^7 iGF particles were incubated in 50 µL of flow cytometry stain buffer containing the required concentration of conjugated monoclonal antibodies (R&D Systems, UK) for 1 h at room temperature. Where conjugated antibodies were unavailable, antibodies were custom conjugated using an APC-Antibody conjugation kit (Solulink, USA). In parallel, iGF particles were also stained with an antibody isotype control. iGF particles were then washed once with PBS and re-suspended in 100–500 µL of PBS.

Fluorescence intensity was measured using FACS Canto II flow cytometer (BD Biosciences, UK) by taking a minimum of 10 000 events at medium flow rate. Fluorescence reference standards (Quantum MESF; Bangs Labs Inc., USA) were run on the same day to determine the mean mass of GF per particle using the net geometric
mean of fluorescence intensity (sample geometric mean minus isotype geometric mean).

2.2.2 Soluble growth factor concentration
Soluble GF concentrations were determined with a Bio- Flex Magpix Multiplex Reader (Bio-Rad Laboratories, UK) using pre-mixed Human Magnetic Luminex assay kits with SCF and GMCSF analytes (R&D Systems, UK). Cell culture media were stored at -20°C until use.

2.2.3 Determination of particle number
Particle number was determined using the particle count setting of an automated cell counter, Countess (ThermoFisher, UK). Final particle concentration was determined using flow cytometry where a fixed volume of sample was analyzed using medium flow rate. Flow cytometry data was analyzed using FlowJo software (Treestar, USA) and cells/mL was generated from the particle population gate.

2.3 Functional assays

2.3.1 TF1 cell proliferation assay
TF1 cells (ATCC, UK) were maintained in culture at a density of 2 × 10^5 to 8 × 10^5 cells/mL with media and GMCSF replenishment every two to three days [2]. Media consisted of RPMI 1640 (phenol red and glutamine free; ThermoFisher Scientific), 10% v/v FBS (Life Technologies), 5 mM Glutamax (ThermoFisher Scientific) and 5 ng/mL carrier free GMCSF (R&D Systems).

Unless otherwise stated, TF1 cells were washed three times in GMCSF free media and starved of GMCSF for 24 h. Either soluble GMCSF (sGMCSF) or immobilized GMCSF (iGMCSF) was supplemented to starved TF1 cells and a growth rate was determined thereafter using flow cytometry.

2.3.2 M07e cell proliferation assay
M-07e cells (Creative Biosystems, USA) were maintained in culture at a density of 4 × 10^6 to 1 × 10^6 cells/mL with media, GMCSF and SCF replenishment every two or three days. Media consisted of RPMI 1640 (phenol red and glutamine free), 10% PBS v/v, 5 mM Glutamax, 5 ng/mL GMCSF and 10 ng/mL SCF.

M07e cells were washed three times in GMCSF free media and starved of GMCSF for 24 h. Soluble or immobilized GMCSF, SCF and TPO were supplemented individually to starved M-07e cells and a growth rate was determined thereafter using absolute cell counts determined by flow cytometry.

2.3.3 Micro-bioreactor TF1 culture
A micro-scale stirred tank bioreactor platform (ambr®, Sartorius Stedim, UK) was used to determine the scalability of the immobilization method. Physiochemical parameters were set to: 100% DO_2 (oxygen delivery), pH 7.4, temperature 37°C, stir speed 350 rpm. Non-sparged vessels were used with 1% antifoam additions (Antifoam C, Sigma Aldrich, UK) performed every 24 h beginning at 0 h. The ambr® was programed to sample from vessels every 3 h, dispensing into a microtiter plate containing 4% paraformaldehyde (Sigma Aldrich, UK) which was then analyzed on a flow cytometer to determine cell and particle number. A complete media change was performed every 48–72 h and cells were reset to a density of 2 × 10^5 cells/mL.

For iGF conditions, particles were retained within the vessel by holding a magnet against the vessel, whilst performing media changes.

2.4 Determination of viable cell number

2.4.1 Flow cytometry
Cell number was determined by flow cytometry. A fixed volume of sample was analyzed using medium flow rate. Flow cytometry data was analyzed using FlowJo software (Treestar, USA) and cells/mL was generated from the cell population gate.

2.4.2 ViCell
The AMBR was programmed to sample cell suspensions every ≈4 h with a two-fold dilution with media to determine viable cell number using a Vicell (Beckman Coulter), an automated trypan blue exclusion method cell counter. An average of 50 images per sample was used to determine viable cell number.

2.4.3 Determination of population doublings
Population doublings per day were determined using the following formula:

\[
\text{population doublings} = \left( \frac{\log_{10}(\text{FD}/\text{SD})}{3.32} \right)
\]

where \(\text{FD} = \text{final density (live cells/mL)}\); \(\text{SD} = \text{seeding density (live cells/mL)}\).

2.5 Statistical analysis
To determine the statistical significance of one variable between two groups, a student’s two-tailed t-Test was performed. To determine the statistical significance of one variable between three or more groups a one-way ANOVA was performed followed by Dunnett’s test for multiple comparisons. To determine the statistical significance of two variables between three or more groups a two-way ANOVA was performed followed by Sidak’s test for multiple comparisons.

For all tests a \(P \leq 0.05\) was deemed significant. All statistical tests were performed using GraphPad Prism 6 (GraphPad Software Inc., USA).
3 Results

3.1 Immobilization and control of surface concentration of growth factors

A series of experiments were conducted to determine the effect of reagent concentrations on GF binding to the streptavidin coated beads. Target GFs were biotinylated via reaction of an NHS group on the biotin-PEG\textsubscript{2000}-NHS with primary amines of the lysine residues in the GF (Fig. 1, step 1). Initially we determined the effect of altering the molar ratio of biotin-PEG\textsubscript{2000}-NHS to lysine residues in the target GFs. It was anticipated that biotin–PEG\textsubscript{2000}-NHS in excess of a 1:1 ratio with target lysine would result in free-biotin being carried forward to competitively blank streptavidin on the particles and therefore reduce GF-biotin bound to the particles, whilst below a 1:1 biotin-PEG\textsubscript{2000}-NHS to lysine ratio the number of biotinylated GFs available in the particle binding step would become a limiting factor (Fig. 1, step 2). In accordance with this, maximum surface yields of three GFs (GMCSF, SCF and TPO) were achieved by using the equivalent biotin-PEG\textsubscript{2000}-NHS molarity to the number of lysine residues within the GF (Fig. 2A). This is a potentially simple mechanism to control surface concentration of iGF.

The absolute concentration of biotin–PEG\textsubscript{2000}-NHS and GF during GF biotinylation (Fig. 1, step 1), at a fixed

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Immobilization of growth factors onto streptavidin functionalized microparticles. The immobilization method consists of a two-step reaction. In step 1, the GFs primary amines react with the NHS terminus of a heterobifunctional PEG molecule, NHS-(PEG)\textsubscript{n}-biotin, to produce a biotinylated GF. Once the reaction is complete, streptavidin functionalized microparticles are added in step 2, allowing the biotin terminus of the biotinylated GF to bind to the streptavidin ligands of the microparticles resulting in immobilization.}
\end{figure}
molar ratio of 1:1 biotin-PEG$_{2000}$-NHS to lysine residues, was evaluated to determine its relationship with the subsequent immobilized surface concentration. Surface concentration of iGF approached saturation at 200 µg/mL of soluble GF (sGF) (Fig. 2B). The relationship between the concentration of sGF and iGF per particle should theoretically be sigmoidal (R$^2$ of sigmoidal line = 0.98 for GMCSF and SCF) presuming the mechanism under pinning this is NHS degradation via hydrolysis at a constant rate and a rate of NHS binding with GF that is proportional to reagent concentration [26]. This presents a complementary method to manipulate immobilized surface concentration and indicates the importance of controlling factors that influence reaction kinetics to achieve reproducible results. GF concentrations of 50 µg/mL were taken forward for particle preparation for functional studies; this utilized approximately 25% of the total GF presentation capacity of the beads.

The benefits of ligand co-signalling may require production systems to co-present two or more GFs on a single particle in controlled relative concentrations. It was hypothesized that if multiple biotinylated GFs at equal concentrations were incubated with the particles (Fig. 1, step 2) the relative bound surface concentrations would be determined by lysine residue number (and therefore biotinylated sites available for streptavidin binding). The
three GFs (GMCSF, SCF and TPO) mixed at equal concentrations, either in pairs or all three, resulted in surface binding of each GF in proportion to its lysine content (SCF>GMCSF>TPO) with a high degree of linear correlation ($R^2 > 0.98$; Fig. 2C). These indicated relative surface concentrations could be controlled via compensating for different lysine residues with changes in GF concentration.

### 3.2 Functionality, stability, and manipulation of immobilized GMCSF in a non-mixed culture system

Following demonstration that GFs could be effectively immobilized at controlled surface concentrations we sought to demonstrate iGFs retained functionality. Furthermore, we aimed to show iGFs could be manipulated independently of the bulk media with the use of magnets and retain prolonged functionality.

The functional activity of iGMCSF was investigated using a GMCSF dependent cell line, TF-1. A dose-response of sGMCSF and iGMCSF was compared to determine the relative response of TF-1. sGMCSF reached a maximal response at approximately 2 ng/mL in accordance with prior studies (Fig. 3A) [25]. iGMCSF created at very low surface concentrations (0.008–0.09 fg/bead) stimulated a linear increase in growth rate with a logarithmic increase in particle concentration, matching the soluble response at approximately $1 \times 10^5$ particles/mL at 0.09 fg/particle or 0.009 ng/mL (Fig. 3Bi). Conversion of the different particle concentrations and surface concentrations into total iGF presented per mL allowed calculation of a single dose.
response (Fig. 3Bi). This indicated a maximal immobi-

lized dose response of approximately 1–10 pg/mL, some

three orders of magnitude more potent than the soluble

equivalent. The formation of a relatively unified dose

response curve from a range of different surface and par-

ticle concentrations suggest modulation of either surface

concentration or particle number can be used to alter

concentration of presented GF to a similar degree.

The functional stability of iGMCSF was tested by cul-
ture of TF-1 cells with a single set of GMCSF coated par-
ticles magnetically retained through serial bulk medium

changes in a static system. A positive control was treated

with a media exchange containing sGMCSF every 48 h; a

negative control had no GMCSF. The cell growth rate for

the iGMSCF and sGMCSF conditions were equivalent for

the experimental duration of 192 h over three media

changes (Fig. 3C). The surface concentration of iGMCSF

was 1.56 fg/particle (or 0.3 ng/mL at 2 × 10^5 particles/mL)
equivalent to a 94% decrease in total GF required to

stimulate the maximal biological response. Including the

time changes the immobilized culture used 1.5% of

the GF over the 192 h relative to the soluble control.

sGMCSF concentration was monitored in all experimen-
tal systems via Magpix™ analysis to confirm no sGF

leached from the immobilized system.

3.3 Functionality, stability, and manipulation of

immobilized GMCSF in stirred tank

bioreactors and effects of transient exposure
to growth factor

The method presented can theoretically be scaled using

surface area (particle number) to volume ratio in a suspen-
sion culture; we therefore aimed to validate the functional

activity of iGMCSF under mechanical agitation in a

micro-scale stirred tank bioreactor system (ambr®). Fur-

ther, we proposed that continuous exposure of GF is

unnecessary, or may have negative effects for continuous
growth [27–28].

TF-1 cells were cultured in the ambr® system over 120 h

with either sGMCSF or iGMCSF. Under stirred condi-
tions the cells responded equivalently to continual exposure

of both sGMCSF and iGMCSF over the 120 h, indicating agi-
tation of the cells/particles was not a barrier to contact and

signaling, and iGMCSF retained functionality over an

extended period (Fig. 4A). At sequential time intervals after

initiation of culture, GF was withdrawn to determine if

stimulation and decay of cell growth was equivalent for

GMCSF in both soluble and immobilized forms. Continued

cell growth was observed for a significant time period after

removal of sGMCSF or iGMCSF, however the duration and

magnitude of growth was dependent on time of exposure

and form of presentation (Fig. 4A). Cell growth measured

at 50 h showed a significantly (P = 0.01–0.05) higher response

than had been achieved with short exposure (2 and 4 h) to

iGMCSF compared to equivalent short exposure to sGF

(Fig. 4B). Similarly, cell numbers continued to significantly

increase (P ≤ 0.05) for a longer period after a short exposure

(2 and 4 h) to iGMCSF compared to the equivalent exposure

to sGMCSF (Fig. 4C). iGMCSF concentrations used

throughout the experiment were calculated to be equiva-

lent to 0.4 ng/mL.

3.4 Functionality and dose response

of immobilized GMCSF, SCF and TPO

The nature of the GF immobilization through lysine

groups could potentially lead to reduced activity if the

binding interfered with active site or induced conforma-
tional change. We therefore determined the functionality

dose-response of iGMCSF, iTPO and iSCF using an

alternative cell line, M-07e, with relevant growth sensitiv-

ty to these GFs.

Both SCF and TPO GFs also remained biologically

active once immobilized (Fig. 5). GMCSF and TPO both

significantly exceeded the maximum sGF response

(P ≤ 0.0001) whereas SCF did not achieve the soluble

response despite reaching the top of a response curve.

Maximum responses were achieved with 2.32 × 10^4 par-
ticles/mL (1.4 fg/particle) for GMCSF, 2.65 × 10^4 particles/

mL for TPO, and 8.42 × 10^6 particles/mL (1.9 fg/particle)

for SCF (Fig. 5). Negative effects on cell viability were

observed for highest concentrations of GMCSF and TPO

(>5.35 × 10^4 and >8.82 × 10^4 particles/mL respectively).

In this cell line 0.003 ng/mL of iGMCSF was required to

accomplish the maximum soluble response achieved

using 10 ng/mL of sGMCSF, providing a similar orders of

magnitude potency gain to that observed in the TF-1 line.

Additionally, 16 ng/mL of iSCF accomplished 65% of the

maximum response achieved with 200 ng/mL of sSCF.

This is consistent with literature indicating distinct roles

for soluble and bound SCF [14, 15]. A single point concen-

tration experiment with immobilized IL-3 showed that

0.0059 ng/mL (± 0.0009 ng/mL) of immobilized IL-3 pro-
duced approximately the same growth response (0.32 PD/
day ± 0.0091 StDev) as 0.1 ng/mL soluble IL-3 (0.34 PD/
day ± 0.011 StDev).

4 Discussion

An approach for incorporating iGF or other proteins into

scalable suspension cell culture systems has been devel-

oped. The method enables significant reduction in the

total quantity of some GFs required to produce a func-
tional response relative to a soluble system. Further it

allows control of local surface GF concentration as well as

bulk GF concentration. The magnetic component of the

particles allows complex temporal profiles of GF presenta-
tion independent from changes in the bulk media. The

iGF appear to display alternate kinetics with respect to

stimulation and decay of function. In concert these prop-
erties provide a platform for cost reduction and increased control in manufacture of complex GF dependent cell systems such as those being developed for haematopoietic products or broader cell therapies.

Multiple GF immobilization methodologies are available. The important criteria for the proposed application are compatibility with clinical grade production, functionality, controllability and economics. The GF immobilization technique retains biological function in three different GFs. Although it is possible that some of the iGF is inactive or unavailable depending on the orientation of binding, quantification suggests that despite such theoretical concerns a large potency and economic gain is realizable through this approach to immobilization and recycling. The immobilization method has the potential for other protein targets important in hematopoietic cell expansion including Notch ligands, Wnt ligands and integrins [29]; this provides an opportunity to recapitulate some surface presented aspects of the stem cell niche in simple and well-mixed scalable systems and control a wider range of biological outcomes. The compatibility of the approach with agitated and well mixed systems is a key criterion; it ensures that particle-cell interactions should controllably scale and incorporates the advantages of spatially homogenous control of factors such as pH and dissolved gasses.

Figure 4. Functionality, stability, and manipulation of immobilized GMCSF in stirred tank bioreactors and effects of transient exposure to growth factor. (A) The effects of immobilized and soluble GMCSF exposure time on subsequent TF1 cell growth rates were investigated in an agitated scalable cell culture bioreactor. Identical cell growth rates were achieved with continuous exposure to immobilized and soluble GMCSF (n = 2 ± SD). Vertical dashed line represents the time of a full media passage and cell reset to initial seeding density. (B) A comparison of cell growth rates at a single time-point from (A) (50 h) for immobilized and soluble GMCSF were investigated. Cell growth was significantly increased at 2 and 4 h of exposure to immobilized GMCSF when compared to soluble GMCSF (n = 2 ± SD). Single asterisk represents a P value of 0.01–0.05. (C) The period of sustained cell growth following growth factor removal was calculated from (A). A significant increase in the sustained cell growth was achieved with 2 and 4 h of exposure to immobilized GMCSF (n = 2 ± SD). Single asterisk represents a P value of 0.01–0.05.
The demonstrated capability to control the surface presentation of iGFs allows the concentration and ratio of GFs to be tailored depending on the required functional response. The importance of spatial presentation of GFs for distinct functional responses has been reported, particularly in T-cell signaling [20]. This property of the system therefore increases the ability to tailor particles to different cell types and specific outcomes. iGF removal is also an important consideration for an economic intensified production system; the increased frequency of bulk media exchange with higher cell density (due to exhaustion of other medium components or build-up of toxic metabolites) will increase the potential cost impact of separating expensive GFs with alternate dynamics. For example, if a GF is subject to decay with time in the reactor, potentially cell mediated, presenting the GF intermittently for 2 h would allow more specific productivity per GF than constant exposure. GMCSF data suggest that some GFs could be effective with particle concentrations in the order of 10³/mL given a suitably high surface concentration, providing further opportunity to reduce cost through minimizing particle use. The biological basis for the difference between iGF and sGF was not determined. However, there is a strong literature base indicating the potency and signaling longevity enhancements could be due to high local concentration of GF on the particles through mechanisms such as receptor multivalency and clustering [12, 30–32]. Short interaction times (50 ms) between enzyme and substrate are reported providing a basis for transient exposure sustaining cell growth [33]. It is also possible, given the extremely high potency, that some particle carry over could lead to sustained growth.

Figure 5. Functionality and dose response of immobilized GMCSF, SCF and TPO. The function and dose-response of three immobilized growth factors were investigated in a multi-factor dependent cell line (M-07e). All three factors remain biologically active once immobilized. Immobilized GMCSF and TPO both significantly exceed the maximal soluble response whereas SCF did not achieve the maximal soluble response. Where quantified, data points are labelled with total quantity of immobilized growth factor (n = 3 ± S.D.). * P = 0.01–0.05, ** P = 0.001–0.01, *** P = 0.0001–0.001.
after particle removal; monitoring particle carry over via flow cytometer indicated greater than 98% removal efficiency. Such a phenomenon would also not explain the observation that time of exposure to iGF had a ‘U-shaped’ relationship with growth response.

In conclusion, we have demonstrated a versatile and scalable technology for controlling iGF presentation in suspension cell culture platforms. The immobilization technique has economic and environmental benefits over soluble equivalents due to significant reductions of some GF quantities required to induce the maximal biological response and ability to recycle GFs independently from other bulk media components. The distinct functional effects of immobilizing GF and control potential of this system will allow more regulated engineering of complex GF dependent cell culture systems.

The authors declare the following potential conflict of interest. Improved cell culture using beads. Patent pending. Filed 19/05/2016. GB1608847.8

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Biotech Method

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Biotech Method

A simplified procedure for antibody engineering by yeast surface display: Coupling display levels and target binding by ribosomal skipping
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Biotech Method

Predictive glycoengineering of biosimilars using a Markov chain glycosylation model
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