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Microscale Biomaterials with Bioinspired Complexity of Early Embryo Development and in the Ovary for Tissue Engineering and Regenerative Medicine

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Abstract

Tissue engineering and regenerative medicine (TERM) are attracting more and more attention for treating various diseases in modern medicine. Various biomaterials including hydrogels and scaffolds have been developed to prepare cells (particularly stem cells) and tissues under 3D conditions for TERM applications. Although these biomaterials are usually homogeneous in early studies, effort has been made recently to generate biomaterials with the spatiotemporal complexities present in the native milieu of the specific cells and tissues under investigation. In this communication, the microfluidic and coaxial electrospray approaches that we used for generating microscale biomaterials with the spatial complexity of both pre-hatching embryos and ovary in the female reproductive system were introduced. This is followed by an overview of our recent work on applying the resultant bioinspired biomaterials for cultivation of normal and cancer stem cells, regeneration of cardiac tissue, and culture of ovarian follicles. The cardiac regeneration studies show the importance of using different biomaterials to engineer stem cells at different stages (*i.e.*, *in vitro* culture *versus in vivo* implantation) for tissue regeneration. All the studies demonstrate the merit of accounting for bioinspired complexities in engineering cells and tissues for TERM applications.

Graphical Abstract

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Keywords

Stem cells; cancer stem cells; follicles; biomimetic 3D culture; core-shell microcapsule

Stem cells including both pluripotent and multipotent ones hold great potential and have been extensively explored in recent years for tissue regeneration and cell-based therapies^{1–12}. However, the difficulty to culture and expand stem cells in large quantities with high stemness and purity *in vitro* has been one of the major hurdles to the wide and safe application of stem cells for treating diseases in the clinic^{1–2,13–15}. This is mainly because the systems used today for *in vitro* culture of stem cells still do not sufficiently recapitulate the *in vivo* microenvironment or niche of stem cells. This insufficiency may result in spontaneous differentiation and/or genetic alterations in stem cells during *in vitro* culture^{16–28}. Therefore, further improvement of the technology of stem cell culture and expansion *in vitro* is of significance to facilitate the wide application and eventual success of the modern stem cell-based medicine.

As shown in Fig. 1, the female reproductive system is the only system in nature that contains all the different types of stem cells including the totipotent one-cell embryo that is known as zygote, the pluripotent embryonic stem cells in the inner cell mass of the blastocyst-stage embryos, and multipotent adult stem cells such as that in the bone marrow of a progeny in the uterus²⁹. Interestingly, all the totipotent-pluripotent stem cells reside in a permissive aqueous core that is enclosed in a semi-permeable hydrogel shell (of glycoproteins) known as the zona pellucida^{30–33}. To further differentiate, the multi-cell aggregate in the core is released out of the zona pellucida (which is known as hatching) and re-encapsulated in the trophoblast for implantation into the uterus wall.

Moreover, the pre-hatching embryos are no more than ~100–150 μm in diameter for humans, and they are no more than ~300–400 μm in diameter regardless of the species from small rodents to large animals such as pigs, dogs, and cows^{25,34–37}. This is probably due to the limited diffusion length (typically ~100–200, equivalent to the radius of mammalian embryos) of oxygen and nutrients in cellularized tissues *in vivo* and the distance between two capillaries in highly cellularized tissues is usually less than ~300–400^{38–44}. The pre-implantation embryos are one of the few tissues/organs that do not have blood vessels in human body.

The aforementioned observations suggest that the spatial complexity of a miniaturized core-shell configuration in pre-hatching embryos, the native home of totipotent-pluripotent stem cells, is desired for culturing stem cells to maintain their stemness. Therefore, we have been working on generating microcapsules with the bioinspired core-shell complexity for stem cell culture and tissue regeneration.

Generation of Microscale Biomaterials Systems with the Bioinspired Core-Shell Complexity

It is worth noting that although many studies have been reported to produce core-shell capsules for drug delivery^{45–48}, they are not suitable for encapsulating living cells due to the necessity of using highly cytotoxic organic solvent in the production procedure. In addition, multi-step approaches have been explored to encapsulate living cells in core-shell microcapsules, they are either too time-consuming or the resultant shell is not made of hydrogel^{49–52}. More recently, several studies were reported to produce microcapsules with a hydrogel shell for cell encapsulation in one step^{53–60}. Here, we focused on two approaches that we developed. The first approach is called coaxial electrospray⁵³, which is schematically illustrated in Fig. 2A. To generate the cell/tissue-laden core-shell microcapsules, aqueous core solution with cells/tissues and aqueous shell solution of sodium alginate are injected into the inner (core) and outer (shell) lumens of a coaxial needle (Fig. 2B), respectively. As a result, the two fluids form core-shell drops at the needle tip and as a result of flow instability and the applied electrostatic voltage (~1.5–2.0 kV), the drops further break up into microscale droplets⁶¹. Sodium alginate solution in the shell is crosslinked (*i.e.*, hardened) to form calcium alginate hydrogel by the aqueous calcium chloride solution once they are in contact in the gel bath⁶². Alginate are used to fabricate the shell of the core-shell microcapsules because of its excellent biocompatibility and reversible gelation with divalent cations such as Ca^{2+} under mild condition that is not harmful to living cells^{62–66}. When needed, the alginate hydrogel shell can be quickly removed using an isotonic solution of sodium citrate to retrieve the cells with no evident damage to the cells^{62–66}. For this approach, the viscosity and flow rate of the two aqueous fluids must be carefully adjusted to produce microcapsules with desired size and core-shell morphology⁵³. However, an analytical or computational model for predicting the coaxial electrospray process is still not available, which warrants further study in the future to guide the refinement of the coaxial electrospray approach for producing core-shell microcapsules encapsulated with living cells.

We have also developed non-planar polydimethylsiloxane (PDMS) microfluidic devices for generating the bioinspired biomaterials systems with an alginate hydrogel shell and a permissive aqueous core encapsulated with cells or tissues for biomimetic 3D culture^{54–56}. A schematic top view of a typical microchannel system is given in Fig. 2C. Figure 2D shows a zoom-in view of the non-planar design of the flow-focusing junction (the boxed region in Fig. 2C), where $H_1 < H_2 < H_3$ for generating the core-shell microcapsules (unlike many planar microfluidic devices, the height is equal for all microfluidic channels). We have fabricated the non-planar microchannel using a multilayer (3-step UV exposure) SU-8 fabrication technique^{54–56}.

To generate the cell/tissue-laden core-shell microcapsules, aqueous core solution with cells/tissues, aqueous shell solution of alginate, and an emulsion of mineral oil and aqueous calcium chloride solution are injected into the device from the I-1, I-2, and I-3 inlet, respectively. At the flow-focusing junction, the aqueous core and shell solutions are pinched into droplets by the oil emulsion flow as a result of the Rayleigh-Plateau instability^{67–68}. Sodium alginate solution in the shell is crosslinked to form calcium alginate hydrogel by the aqueous calcium chloride solution in the oil emulsion once they are in contact, but mainly during traveling in the downstream serpentine channel^{54,62}. The aqueous core flow is arranged in the center of alginate shell flow both horizontally and vertically (Fig. 2C–D).

We further designed an extraction channel at the downstream of the serpentine channel (Fig. 2C) to achieve efficient on-chip extraction of the cell/tissue-laden microcapsules from the oil emulsion into an isotonic aqueous solution introduced into the device through inlet I-4. This is achieved by utilizing surface tension force and/or dielectrophoresis (DEP) force to overcome the viscous force in the microfluidic flow and move the cell/tissue-laden microcapsules from the oil emulsion into the isotonic extraction solution^{55,69–70}.

Because the two aqueous core and shell solutions could easily mix in the flow-focusing junction to produce microcapsules with no clear core-shell configuration⁷¹, it could be time-consuming to generate the bioinspired microcapsule system by the trial-and-error process of experimental studies alone. Therefore, it is desired to establish a computational fluid dynamics (CFD) model capable of predicting the complex multiphase microfluidic flow in the non-planar microfluidic flow-focusing junction for fabricating the bioinspired microcapsule system. Although computational studies have been conducted to model droplet generation^{71–76}, none has investigated the use of two aqueous flows with direct contact as the dispersed phases for producing core-shell structured hydrogel microcapsules in one step using the non-planar flow-focusing mechanism. This model is very useful for understanding the fundamental physics of the complex multiphase microfluidic flow, which could greatly facilitate the generation of microcapsules with the desired spatial complexities.

In general, the multiphase flow should satisfy the laws of mass and momentum conservation. The mass conservation of an incompressible flow without mass generation is governed by the continuity equation as follows^{77–79}:

$$\nabla \cdot \vec{u} = 0 \quad (1)$$

where $\vec{u}$ is velocity vector, and ∇ is Del operator. The conservation of momentum in an incompressible flow is described by the following Navier-Stokes equation^{77–79}:

$$\rho \frac{\partial \vec{u}}{\partial t} + \rho (\vec{u} \cdot \nabla) \vec{u} = \nabla \cdot [-p\mathbf{I} + \mu(\nabla \vec{u} + (\nabla \vec{u})^T)] + \vec{f}_b \quad (2)$$

where ρ is density, t is time, p is hydrostatic pressure, $\mathbf{I}$ is identity matrix, μ is dynamic viscosity, the superscript T represents transpose, and $\vec{f}_b$ is volumetric body force. The most common body force is gravity. However, in the flow of most microfluidic devices including

the one for this project, the ratios of gravity to viscous and interfacial tension forces are far less than 1 and can be neglected^{69,71}.

The interfacial tension force is treated as an equivalent body force when using the level set method to track the interface with the level set function (ϕ) defined by the following equation^{80–82}:

$$\frac{\partial \phi_i}{\partial t} + \vec{u} \cdot \nabla \phi_i = \gamma_{ls} \nabla \cdot (\epsilon_{ls} \nabla \phi_i - \phi_i (1 - \phi_i) \frac{\nabla \phi_i}{|\nabla \phi_i|}) \quad (3)$$

where the subscript i represents the i th level set function, and γ_{ls} and ϵ_{ls} are level set parameters that can be adjusted to stabilize the numerical calculation without affecting the converged modeling results^{80–81}. The number of level set functions needed is equal to the number of phases minus one. The level set function ϕ_i equals to 1 for the i th phase and 0 for the other phases.

With ϕ_i , the interfacial tension force can be calculated as follows^{71,83}:

$$\vec{f}_b = \vec{f}_{it} = \nabla \cdot (\sigma (\mathbf{I} - (\vec{n} \vec{n}^T))) \delta \quad (4)$$

where σ is interfacial tension (N/m), $\vec{n} (= \nabla \phi_i / |\nabla \phi_i|)$ is unit outward normal vector of interface, and $\delta (= 6 |\nabla \phi_i| \phi_i (1 - \phi_i))$ is Dirac delta function that is nonzero only at the interface.

We have conducted studies assuming a planar design (*i.e.*, H1, H2, and H3 are equal rather than different in Fig. 2D) and using mineral oil as the continuous carrier fluid, to study the effect of the viscosity of the two aqueous phases (assuming they have the same viscosity) on the droplet morphology⁷¹. The model for the planar design was verified by comparing the predicted size and shape of the core and shell of microcapsules with that from experimental studies (Fig. 3A). As shown in Fig. 3B, when the two aqueous fluids are less viscous, it is more difficult to form the core-shell morphology because of mixing between them. In addition, the flow pattern evolves from jetting to dripping mode with the decrease of viscosity, which is consistent with that reported elsewhere⁸⁴. The decrease in viscosity results in the decrease in capillary number ($= \mu u / \sigma$), the ratio of viscous to interfacial tension force. As a result, interfacial tension force dominates, which tends to break up the aqueous flows leading to the dripping mode while more viscous flows tend to be in the jetting mode. Our modeling results also show that letting the two aqueous flows meet at a distance (d_s , Fig. 3B) before touching the oil flow affects the morphology of the resultant droplets. With further development to account for the nonplanar design, the model will be a valuable tool for systemically studying the impact of the flow-focusing design and fluid properties on the resultant droplet/microcapsule morphology.

Biomimetic 3D Culture of Stem Cells Using Core-Shell Microcapsules

We have cultured mouse embryonic stem cells (ESCs) in the bioinspired core-shell biomaterials system for biomimetic 3D culture^{53–54}. Typical phase and fluorescence images of the bioinspired core-shell and conventional homogeneous microcapsules encapsulated with the ESCs immediately after encapsulation (day 1) showing single mouse ESCs with high viability in the microcapsules are given in Fig. 4A. After 7 days of culture, the cells in the bioinspired microcapsules proliferated to form a single aggregate containing 482 ± 14 cells in each of the microcapsules, which resembles the morula stage of pre-hatching embryos where a single aggregate of totipotent-pluripotent stem cells is enclosed in a semi-permeable hydrogel-like shell (zona pellucida, Fig. 1). In contrast, multiple aggregates of uncontrolled size and shape together with many dead cells are observable under the conventional 3D culture in homogeneous alginate microcapsules.

To investigate the undifferentiated properties of the ESCs, further quantitative RT-PCR (qRT-PCR) analyses were conducted for three common stemness genes (Klf2, Nanog, and Oct-4), as well as five differentiation genes including Nestin for ectoderm, Sox-7 for endoderm, Brachyury (or T) for mesoderm, Nkx2.5 for early cardiac commitment, and cTnT for late cardiac differentiation. As shown in Fig. 4B, the bioinspired 3D culture can maintain significantly higher expression of the stemness genes (except Oct-4 that should be equally high for stem cells of the same types) and significantly lower expression of all the differentiation genes than the conventional 3D and 2D culture methods.

Future studies in this direction will be to apply the bioinspired complex microcapsule systems for culturing various induced pluripotent stem cells (iPSCs) and multipotent (bone marrow and adipose-derived) stem cells^{61,85–88}, as compared to the conventional 3D and 2D culture methods in maintaining their stemness and preventing their spontaneous differentiation.

Cardiac Regeneration Using Microscale Biomaterials Systems with Bioinspired Spatial and Temporal (Spatiotemporal) Complexities

It is difficult to achieve minimally invasive injectable cell delivery while maintaining a high cell and animal survival *in vivo* for stem cell therapy (SCT) of myocardial infarction (MI), the leading cause of death in the United States and globally^{89–92}. We took a bioinspired procedure mimicking that used by nature (Fig. 1) to prepare totipotent-pluripotent stem cells for implantation in the uterus, to address this grand challenge using biomaterials systems with the bioinspired spatial and temporal complexities²⁹. As shown in Fig. 5A, this is achieved by forming 3D microscale aggregates of ESCs in core-shell microcapsules, pre-differentiating the aggregated cells to the early cardiac lineage^{53,93–94}, releasing them out of the core-shell microcapsules, and re-encapsulating the aggregates (Bare-A) in a biocompatible and biodegradable micromatrix of alginate and chitosan (ACM)⁴⁹ without significantly altering their size for injectable delivery. After intramyocardial injection into the MI heart (Fig. 5B), the ACM encapsulated aggregates (ACM-A) produced with this bioinspired procedure significantly improves the survival of the injected cells by more than 6 times (7.3 *versus* 47.0%) compared to the conventional practice with injecting single cells

(Fig. 5C), minimizes inflammatory responses to the injected cells (Fig. 5D), and eliminates the notorious issue of teratoma formation associated with directly injecting pluripotent stem cells²⁹. As a result, it significantly reduces fibrosis (Fig. 5E), facilitates cardiac regeneration (Fig. 5F), and ultimately significantly enhances the survival of animals with MI (Fig. 5G)²⁹.

Of note, the bioinspired procedure for preparing totipotent/pluripotent stem cells requires not only spatial but also temporal complexities. More specifically, a pre-hatching embryo-like core-shell configuration was used for culturing the stem cells to maintain their stemness, while a micromatrix (ACM) was used to encapsulate the pre-differentiated stem cell aggregate for implantation and further differentiation. The latter is called inverse scaffold engineering because for conventional scaffold engineering, cells are seeded into a pre-made acellular scaffold²⁹. The inverse scaffold engineering enables fabrication of microscale ($\sim 125\ \mu\text{m}$) constructs (ACM-A) densely packed with ~ 1500 cells per construct, which is difficult (if not impossible) to achieve with the conventional scaffold engineering approach²⁹.

Future studies in this direction will be to determine the mechanisms responsible for the significantly improved cell and animal survival and to test the approach with other types of stem cells including iPSCs and multipotent (bone marrow and adipose-derived) stem cells. Moreover, the temporal complexity of the natural procedure of preparing the totipotent-pluripotent stem cells for implementation is cell-controlled²⁹. Designing biomaterials that can be controlled by the encapsulated cells to mimic the temporal complexity would be of interest.

Isolation/Enrichment of Cancer Stem Cells Using Core-Shell Microcapsules

Cancer stem cells (CSCs) or tumor initiating cells (TICs) are highly tumorigenic and a major cause of cancer metastasis^{95–96}. Moreover, the CSCs are highly resistant to the commonly used chemotherapy drugs, resulting in cancer recurrence after the conventional chemotherapy^{97–99}. Therefore, the CSCs have attracted a great deal of attention lately in the field of cancer research¹⁰⁰. One of the major challenges of working with the CSCs is the difficulty to isolate/enrich them out of the bulk cancer cell population where they usually take up less than 5%. In view of the exceptional capability of our microcapsule system with the bioinspired spatial complexity of core-shell configuration in maintaining the stemness of normal stem cells (Fig. 4), we are interested in using the bioinspired system to isolate/enrich the CSCs. Indeed, with the bioinspired system to encapsulate ~ 40 PC-3 prostate cancer cells in the core for miniaturized 3D culture for only 2 days in cancer stem cell medium, the resultant cells are much more tumorigenic than both the parent PC-3 cells without enrichment and the cells from the conventional approach of homogeneous bulk suspension culture in ultra-low attachment for either 2 or 10 days in the same medium (Fig. 6A–B)¹⁰¹. The 10-day culture is commonly used by the conventional approach for enriching CSCs. could greatly facilitate the application of the personalized medicine to treat cancer *via* targeting the CSCs in the clinic.

Future studies in this direction include testing this approach using multiple cell lines, as well as cancer cells from tumors collected from patients. It is also important to identify the

molecular mechanisms that facilitate the isolation/enrichment of CSCs in the miniaturized biomimetic milieu. The significance of both isolating/enriching the CSCs and reducing the time for isolating/enriching the CSCs from 10 to 2 days is tremendous. This is because they makes it possible to develop patient-specific strategy to kill the CSCs and eliminate cancer recurrence and metastasis that are the major causes of cancer-related mortality.

Biomimetic 3D Culture of Ovarian Follicles in Core-Shell Microcapsules

According to the Centers for Disease Control and Prevention (CDC), impaired reproduction/fertility affects millions of reproductive-age American women today¹⁰². The ovary is a critical organ for female reproduction or fertility, and the ovarian follicles are the fundamental functional tissue unit of the ovary and develop continuously (left, Fig. 7A). For humans, females are born with a total of $\sim 10^6$ follicles that degenerate over time^{103–107}; Less than $\sim 30\%$ of the follicles could survive to puberty, and it continues to decline during adulthood (particularly after ~ 35 years old) to the point of extinction at the age of ~ 50 . Moreover, many women may not ovulate fertilizable oocytes as result of ovarian disorders that are either genetic (*i.e.*, born with) or acquired after exposure to gonadotoxic environmental/occupational hazards and/or medical treatments (*e.g.*, radio and chemotherapies)^{108–115}. Therefore, isolation of ovarian follicles for *in vitro* culture to obtain oocytes has been regarded as a promising strategy for restoring the female fertility^{108–115}.

However, none of the contemporary 2D or homogeneous 3D culture systems recapitulate the native milieu in which the growing follicles span both the harder cortex and softer medulla (*i.e.*, the mechanical heterogeneity) during their development in the ovary *in vivo* (left, Fig. 7A)^{55–56,116}. Using the microfluidic device showing in Fig. 2C–D, we have fabricated complex ovarian microtissue system that recapitulates the spatial complexity for biomimetic 3D culture of ovarian follicles (right, Fig. 7A)^{55–56}. Typical images showing an early secondary pre-antral follicle in the biomimetic ovarian microtissue with a soft core of 0.5% collagen (Col) and harder shell of 2% alginate (Alg) on day 0, its development to the antral stage, and the subsequent ovulation are given in Fig. 7B. The fibrous collagen core in the alginate shell is evident on early days and disappeared in later days due to its biodegradable nature, indicating the temporal complexity of the bioinspired culture system. It is worth noting that this is the first ever study that achieved biomimetic ovulation *in vitro*. Moreover, the ovulation can occur in the absence of luteinizing hormone (LH) and epidermal growth factor (EGF) (Fig. 7C). LH and EGF are indispensable for ovulation in the absence of the spatial complexity^{117–122}. The development is nearly 0 in nine days under the conventional 2D (Fig. 7D) or homogeneous 3D culture. These data indicate the crucial role of the spatial complexity in the ovary in determining fertility, which is consistent with the literature suggesting that disruption of the normal physical microenvironment in the ovary may cause ovarian disorders such as premature ovarian failure (POF) and polycystic ovary syndrome (PCOS)^{123–124}.

Future studies in this direction will determine the optimal core and shell materials to improve the percentage of development to the antral stage from 25% to more than 90%, and to understand the molecular mechanisms underlying the action of the spatial complexity in

the bioinspired 3D culture system for *in vitro* culture of not only pre-antral but also primary follicles.

SUMMARY

In summary, both computational and experimental approaches have been taken to explore the generation of complex biomaterials systems with the complexities inspired by nature and the complex systems have been applied to address important biomedical problems. We are particularly interested in the complexities of tissues in the female reproductive system because it is the only system in nature that contains all the different types of stem cells from totipotent (*i.e.*, the one-cell embryo or zygote), to pluripotent (*e.g.*, the embryonic stem cells in the blast-stage embryos) and multipotent (*e.g.*, mesenchymal stem cells in the bone marrow of a progeny in the uterus) cells. Our studies show the spatial complexities present in the various stages of embryos are important to maintain the stemness of pluripotent stem cells and preparing (including both culturing and differentiating) pluripotent stem cells for cardiac tissue regeneration to treat myocardial infarction. The spatial complexity in the pre-hatching embryos also facilitates the enrichment of CSCs for developing patient specific strategies to treat cancer by eliminating the CSCs that are resistant to the conventional radio- and chemotherapies. The spatial complexity in the ovary is crucial for developing early pre-antral follicles to the antral stage and ovulation of the antral follicles. It is worth noting that microcapsules with a core-shell configuration have also been shown to be beneficial for engineering islets to implant and treat diabetes⁶⁰. Besides the aforementioned potential future studies in previous sections, it is also desired to build complex network models for accurately predicting the emergence of the complex biomaterials systems^{125–126}. All these studies are invaluable to facilitate the widespread application of the bioinspired complex systems approach in the field of biomaterials research for various biomedical applications including tissue engineering and regenerative medicine.

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Figure 1.

A schematic illustration of the female reproductive system, the only system in nature, that contains all the different types of stem cells including totipotent, pluripotent, and multipotent cells. Also shown are the spatial and temporal complexities that nature uses to prepare the stem cells for implantation into the uterus. This includes the core-shell configuration of the pre-hatching embryos for maintaining their stemness during proliferation to form a multi-cellular aggregate, together with the procedure of pre-differentiation, hatching out of zona pellucida, and re-encapsulation in the trophoblast before implantation into the uterus. This figure is adapted from reference 29 with permission from the author (i.e., Xiaoming He) of the reference.

Figure 2.

Approaches for generating microscale biomaterials systems with the bioinspired core-shell configuration: (A), a schematic overview of the entire coaxial electrospray setup; (B), a zoom-in view of the coaxial needle in the setup; (C), an overview of the entire microfluidic channel system; and (D), a zoom-in view of the microfluidic flow-focusing junction (FFJ). This figure is adapted from reference 53 with permission from The Royal Society of Chemistry (for panels A and B) and from reference 55 with permission from Elsevier (for panels C and D).

Figure 3. Computational study of the multiphase flow in planar microfluidic flow-focusing junction: (A), model verification with droplets of different shapes and (B), effect of viscosity (μ) of the two aqueous (Aq.) phases and the flow-focusing design (*e.g.*, varying d_s) on the droplet morphology. This figure is adapted from reference 71 with permission from The Royal Society of Chemistry.

Figure 4. Mouse embryonic stem cells under the bioinspired 3D culture in the complex core-shell microcapsules *versus* conventional 3D culture in homogeneous microcapsules and 2D culture: (A), viability and morphology and (B), gene expression. *: $p < 0.05$. This figure is adapted from references 53 and 55 with permission from The Royal Society of Chemistry.

Figure 5.

Cardiac regeneration to treat myocardial infarction (MI) using microscale biomaterials systems with bioinspired spatiotemporal complexities: (A), the bioinspired procedure for preparing pluripotent stem cells (*e.g.*, ESCs) for injection showing the spatial and temporal complexities of the biomaterials systems used; (B), a schematic showing the MI created by LAD ligation; (C), survival of the injected cells prepared with the bioinspired procedure (ACM-A) *versus* that without re-encapsulation (Bare-A) and the conventionally used single cells; (D), the bioinspired procedure eliminates strong inflammatory responses that are seen with the Bare-A and single cell treatments (the arrows indicate granulomas consisting of mainly inflammatory cells); (E), the bioinspired procedure significantly reduces fibrosis; (F), regeneration of cardiomyocyte-like cells with striated patterns from the injected cells expressing green fluorescence protein (GFP) in ACM-A indicated by cardiac markers cTnI, Connexin 43, and α -actinin; and (G), significantly improved animal survival using stem cells prepared with the bioinspired procedure (*i.e.*, ACM-A). *: $p < 0.05$. This figure is adapted from reference 29 with permission from the author (*i.e.*, Xiaoming He) of the reference.

Figure 6. Microscale biomaterials systems with the bioinspired spatial complexity of core-shell configuration are exceptional for enriching cancer stem cells (CSCs): (A), growth curve and (B), real image of tumors collected at the last day of experiment, showing tumorigenicity of the CSCs enriched using the systems with ~40 cells in each microcapsule. **: $p < 0.01$. This figure is adapted from reference 101 with permission from Elsevier.

Figure 7. Bioinspired biomaterials systems with spatial complexity for biomimetic 3D culture of ovarian follicles: (A), a schematic illustration of ovarian anatomy showing the mechanical heterogeneity and the biomimetic ovarian microtissue system that recapitulates the heterogeneity; (B), typical images showing the development of the pre-antral follicles in the complex biomimetic 3D culture system; (C), quantitative data showing the effect of luteinizing hormone (LH) and epidermal growth factor (EGF) on the biomimetic ovulation; and (D), typical images showing degeneration of the ovarian follicles under the conventional 2D culture.). This figure is adapted from reference 55 with permission from Elsevier.