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Review

Bone tissue engineering bioreactors: Dynamic culture and the influence of shear stress

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ABSTRACT

A bone tissue engineering strategy involving the *in vitro* expansion of cells on a scaffold before implantation into the body represents a promising alternative to current clinical treatments. To improve *in vitro* culture, bioreactor systems have been widely researched for bone tissue engineering purposes. Spinner flask, rotating wall bioreactors, and perfusion systems have all been the focus of experiments, and each system has advantages and disadvantages. This review seeks to summarize these efforts and provide the current status of research in this area. Research using spinner flasks and rotating wall bioreactors is discussed, but focus is placed on perfusion bioreactor systems. While spinner flasks and rotating wall bioreactors have been shown to improve *in vitro* culture conditions by increasing homogeneity of nutrients in the media, perfusion systems expose cells to shear stress and more efficiently enhance nutrient transfer. Enhanced mineralized matrix deposition and enhancement of osteoblastic signal expression in response to culture in these systems have been widely reported. This review provides analysis of the causes of these changes in signal expression as well as reports on bioreactor systems that have been commercialized.

Contents

Introduction

Every year, over six million bone injuries occur in the United States, and approximately one million bone grafting procedures are performed [1]. The source of bone for these grafts is either from the patient's own body in the case of an autograft or from a cadaver in the case of an allograft. Unfortunately, both of these methods have significant disadvantages. The incidence of medical complications arising after surgery involving an autograft from the iliac crest is nearly 30% [2]. Allografts are subject to an immune response and may transmit disease [2,3]. Since these traditional means of treating bone injuries are associated with limitations, a tissue engineering approach to replace damaged bone represents a promising alternative. A tissue engineering approach involves seeding and growing a cell source on a scaffold and implanting the scaffold and cells into the injury site [4]. Before implantation into the body, the cell containing constructs are often cultured *in vitro* to increase cell proliferation on the scaffold and to allow for differentiation of the stem cells into osteoblasts. However, *in vitro* culture techniques of 3D tissue engineering scaffolds have nutrient transfer limitations that must be overcome to increase the feasibility of cell-based tissue engineering strategies. Bioreactor systems are used to alleviate this nutrient transfer limitation by continuously mixing media and by convectively transporting nutrients to cells.

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In the overall cell-based bone tissue engineering strategy of expanding a stem cell source *in vitro*, culturing and differentiating this cell source on a three-dimensional scaffold, and implanting this scaffold *in vivo*, bioreactors can be used to enhance *in vitro* culture steps. Bioreactors utilize materials and cells that have already been proven effective for bone tissue engineering including polymer scaffolds that are biodegradable and mesenchymal stem cells (MSCs), a population of cells that exists in the bone marrow capable of differentiating into osteoblasts, chondrocytes, and adipocytes (see Table 1 for list of abbreviations) [5,6]. This population represents only a small percentage of cells found in the bone marrow, thus expanding MSCs to clinically relevant numbers represents a significant hurdle to the implementation of a tissue engineering strategy utilizing these cells. In addition to a readily available cell source, the use of biodegradable scaffolds is also of importance as ideally scaffolds degrade *in vivo* and are replaced by new bone, healing the defect without a permanent scaffold presence. Since bones are load bearing, this degradation–regeneration balance is exceptionally important as the scaffold cell construct must provide continuous structural support. Bioreactors have been shown to be used to improve cell seeding efficiency [7–9], cell proliferation [10–13], and mesenchymal stem cell osteoblastic differentiation [14–20]. In addition to enhancing differentiation and proliferation, perhaps the most notable contribution of bioreactor systems to a bone tissue engineering strategy is the possibility of automation. A clinically relevant strategy must greatly minimize the risk of contamination from bacteria and other cells, reduce labor intensity, and reduce costs associated with *in vitro* cell culture. Bioreactor systems have the potential to minimize all of these aspects through automated cell culture. A cell source could be added to a bioreactor, seeded using the bioreactor, and cultured continuously in the closed system. Nutrient and oxygen concentrations could be monitored by the system and media changes could be automated. By reducing the potential for contamination and the labor intensity bioreactors could eventually greatly improve the feasibility of bone tissue engineering strategies. Continued research both on developing new innovative bioreactor systems and using established systems to determine relationships between system parameters and cell proliferation and differentiation should be completed to bring this to fruition.

In addition to the possibility of automation, bioreactors can improve *in vitro* cell culture. *In vitro* cell growth is especially hindered in three-dimensional scaffold culture of these cells. In these scaffolds, nutrient gradients develop in static culture where the cells at the surface are consuming oxygen, glucose, and other nutrients faster than their replacement by diffusion. This creates a gradient where cells nearer to the surface of the scaffold receive adequate nutrients, but the concentration of these nutrients decrease toward the center of the scaffold. Cell death then occurs at the center of the scaffold as nutrient and oxygen concentrations drop below the minimum necessary to sustain cell growth [21]. In the case of a bone tissue engineering construct in which cells are producing matrix, this gradient is magnified as the matrix produced by cells on the exterior portion of the scaffold further reduces nutrient transfer. To mitigate this hurdle bioreactor systems have been developed to optimize *in vitro* culture conditions. A bioreactor is a culture system designed to support or expand a population of cells through dynamic culture and a controlled environment. This definition provides for a wide array of designs that would qualify as bioreactors, but this review focuses on three classes of bioreactor systems that have been widely utilized in bone tissue engineering: spinner flasks [22–24], rotating wall [22–26], and perfusion systems [16,19,27–31]. Each of these bioreactor types has been demonstrated to be an effective means to culture cells for bone tissue engineering purposes. Spinner flask and rotating wall bioreactor systems are effective at creating a homogenous media solution on the exterior of the scaffold but do not effectively perfuse media into the scaffold. Perfusion systems have been demonstrated to effectively perfuse media throughout the scaffold and have been shown to upregulate osteoblastic markers and increase calcium deposition. Emphasis is placed on perfusion systems as these systems are more complex than spinner flasks and rotating wall bioreactors and feature a variety of designs. Bioreactor systems and perfusion systems in particular enhance nutrient transport and expose cells to fluid shear stresses.

An important aspect of bioreactor systems is their ability to create an *in vitro* environment that is more like the *in vivo* environment of bone [32]. Although bioreactor systems cannot replicate this environment, mechanical stresses and improved nutrient transport aid in improving *in vitro* cell culture. For example, limited transport of nutrients in static culture is in contrast to the *in vivo* conditions of bone as it is a vascular tissue. Because of this *in vitro* nutrient transfer should be improved to optimize culture of cells in three-dimensional scaffolds. Bioreactor systems overcome these barriers via dynamic culture which convectively transports nutrients and exposes cells to mechanical stress. Mechanical stimulation through fluid shear stresses has been shown to be influential on bone differentiation and mineralization [14,27,32]. *In vivo* bone constantly remodels in response to mechanical stresses. It is hypothesized that *in vivo*, these stresses are mainly transmitted to bone cells via fluid shear stresses [33]. As load is applied to bone, interstitial fluid flows through pores in the bone, and the shear stress is sensed by terminally differentiated osteoblasts known as osteocytes. The matrix network around these osteocytes may allow for communication with osteoblasts and osteoprogenitor cells. It is estimated that in response to loading, bone cells experience *in vivo* shears from 8 to 30 dyn/cm^2 [34,35]. Osteoblasts and MSCs have also been shown to directly respond to shear stress [14,15,31,36–39]. Based on the natural environment of bone, an optimal *in vitro* culture system should provide for adequate nutrition and oxygen to cells throughout the scaffold. Furthermore, just as cells respond *in vivo* to fluid shear stress, *in vitro* shear stresses also affect bone cells. This review seeks to highlight experiments that demonstrate the effects of both of these as well as provide some comparison between various perfusion systems in terms of shear stresses.

Table 1
Abbreviations.

ALP	Alkaline phosphatase
BMP	Bone morphogenic protein
BMSC	Bone marrow stromal cell
BSP	Bone sialoprotein
CT	Computed tomography
COX	Cyclooxygenase
ECM	Extracellular matrix
ERK	Extracellular signal-regulated kinase
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
HA	Hydroxyapatite
hASC	Human adipose-derived stem cell
hMSC	Human mesenchymal stem cell
MAPK	Mitogen-activated protein kinase
MSC	Mesenchymal stem cell
OC	Osteocalcin
OPN	Osteopontin
PCL	Polycaprolactone
PLG	Poly(L-lactic-co-glycolide)
PLGA	Poly(lactic-co-glycolic acid)
PLLA	Poly(L-lactic acid)
PGA	Polyglycolic acid
ROBS	Rotating oxygen-permeable bioreactor system
Runx2	Runt-related transcription factor-2
TCP	Tricalcium phosphate
TGF	Transforming growth factor
VEGF	Vascular endothelial growth factor

Spinner flasks and rotating wall bioreactors

A simple bioreactor system to achieve thorough media mixing is the spinner flask (see Table 2 for a summary of spinner flask and rotating wall bioreactor studies). Spinner flasks are composed of a

glass media reservoir with side arms that can be opened to remove scaffolds and media and often have porous covers to allow for gas exchange (Fig. 1). The flask has a stir bar or other stirring mechanism that stirs the media in the flask. Scaffolds are typically suspended from the top of the flask using needles or thread [23,40,41]. Spinner flasks are often used in the culture of cells for bone tissue engineering as they have been shown to increase expression of early osteoblastic marker alkaline phosphatase (ALP), late osteoblastic marker osteocalcin (OC), and calcium deposition as compared to static culture and rotating wall bioreactors [41]. This effect is thought to result from the convective transport of nutrients to the surface of the scaffold in spinner flask culture in contrast to the purely diffusional transport in static culture. This will then increase concentrations of oxygen throughout the scaffold. In static culture, a nutrient concentration gradient can form where cells in the center of the scaffold receive an insufficient supply of nutrients. A nutrient gradient may still exist in spinner flask culture as matrix deposition of rat marrow stromal cells induced into an osteoblastic lineage has been shown to be concentrated on the exterior portions of the scaffold in both spinner flask and static culture [41]. This result was also observed when human mesenchymal stem cells (hMSCs) were cultured for 5 weeks on collagen scaffolds in spinner flasks and bone formed only in the outer 0.5–1.0 mm of 11-mm-diameter scaffolds [22]. The authors of this review speculate that in larger scaffolds such as these, spinner flask culture does not adequately enhance mass transport and a sharp nutrient gradient results leading to cell death in the center of the scaffold, resulting in confinement of matrix to exterior portions of the scaffold. These scaffolds had interconnected pores and were only 1.5 mm thick, but the penetration depth of transport in spinner flasks appeared to be limited to 1.0 mm or less. Despite these results, spinner flasks may also expose cells at the surface of constructs to shear stress which could also aid in enhancing osteogenic differentiation [24].

hMSCs cultured in spinner flasks for 84 days showed similar trends in osteogenic behavior, but both proliferation and differentiation appeared to be accelerated in spinner flask culture [42]. For clinical relevance *in vitro*, culture times should be reduced far below 84 days to decrease costs and the time the patient must wait for an implant. The differences in proliferation and differentiation observed between spinner flask and static culture could be caused by increased nutrient transfer or exposure to shear stresses. Along these lines, culture conditions of spinner flask systems have been shown to affect stem cell differentiation and proliferation [43]. Not only was the expression of ALP higher in spinner flask culture but also the expression was influenced by the rate of stirring. This could indicate that the increased shear resulting from higher stirring rates affects the osteoblastic differentiation of the rat bone marrow stromal cells (BMSCs) used in the study. This increased ALP expression in response to shear may explain the results of another study which compares perfusion culture to spinner flask culture [44]. Rat BMSCs seeded on poly (glycolic acid) (PGA) scaffolds exhibited higher ALP expression levels in spinner flask culture as compared to static culture, but this expression was significantly higher in a perfusion system. In addition to stirring speed, material properties such as pore size have been shown to affect mesenchymal stem cell ingrowth and differentiation [11]. Immortalized hMSCs exhibited a faster rate of differentiation as shown by ALP expression in 200 μm hydroxyapatite (HA) scaffolds as compared to 500 μm pore size scaffolds. Cell proliferation was slightly higher in the 500 μm pore size group, though limited proliferation was observed throughout the study. As pore size has also been shown to affect stem cell differentiation in static culture, it is not surprising that this effect is also observed in spinner flask culture; however, dynamic culture adds additional variables which may affect osteogenic differentiation beyond what is seen in static culture. Despite being a simple system, several studies have shown spinner flasks to support mesenchymal stem cell expansion and osteoblastic differentiation.

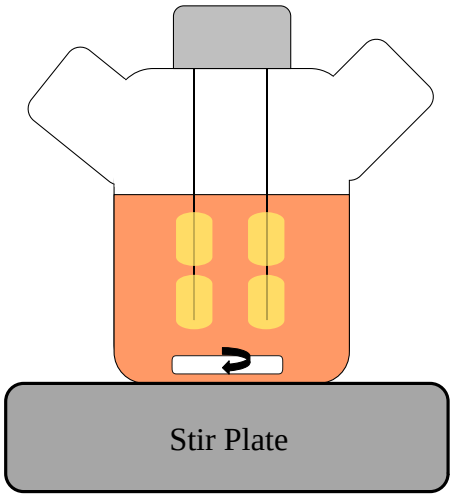

Fig. 1. Schematic of spinner flask bioreactor. Scaffolds are suspended in culture while media is circulated using a stir bar.

Table 2
Studies utilizing rotating wall bioreactors and spinner flasks for bone tissue engineering.

Scaffold material	Effects on osteoblastic differentiation	Reference
Coralline HA	Spinner flask improves cell distribution, proliferation, and osteoblastic differentiation of hMSCs	[11]
	Cells in 200 μm pore size scaffolds differentiated faster than 500 μm scaffolds	[11]
PLGA	Spinner flask cultured hMSCs have higher DNA content and 10-fold increase in calcium deposition after 21 days	[23]
	Rat MSC calcium production 30-fold higher in spinner flask than rotating wall bioreactor	[41]
	Rat MSC-derived osteoblasts mineralized throughout scaffold after rotating wall culture	[49]
Silk	84 days of culture of hMSCs in 15 mm × 5 mm scaffolds showed greater calcium deposition in spinner flask	[42]
PLG	Higher expression of ALP, osteopontin, and calcium deposition in rat osteoblasts cultured in spinner flask compared to rotating wall	[25]
	Developed a microcarrier system to culture osteoblasts in rotating wall system	[46–48]
Bio-derived bone	Rat osteoblasts proliferate slower and produce less bony nodules in spinner flasks as compared to rotating wall	[51]
Gelatin/hyaluronic acid	Histology shows stronger staining of type I collagen, ALP, and osteocalcin in hMSCs after 21 days of culture in spinner flask compared to static and rotating wall	[24]
PCL	Rat MSCs cultured for 4 weeks in rotating wall bioreactor and implanted in rats for 4 weeks. ECM, mineralization, and type I collagen detected throughout the scaffold	[12]
	Rat MSCs showed mineralization and type I collagen after 4 weeks of culture in rotating wall bioreactor	[50]
Collagen	hMSCs express increase amounts of ALP and deposit increased amounts of calcium in spinner flask compared to perfusion and static	[22]
PLGA	Higher ALP activity and cell uniformity in flow perfusion system compared to spinner flask and rotating wall, similar osteocalcin expression and cell numbers in all three systems	[45]
PGA and collagen	Increased OC, ALP, and cell proliferation in spinner flask compared to static, decreased compared to perfusion	[44]
Polyethylene terephthalate	RPM of stirrer increases osteogenic differentiation and proliferation from 10 rpm to 100 rpm in rat BMSCs	[43]

However, matrix production is still observed to be restricted to the exterior of the surface. MSC culture may benefit more from systems that provide direct perfusion of nutrients and expose the cells to greater shear stresses.

Another system used in bone tissue engineering to enhance media mixing is the rotating wall bioreactor. The design features two concentric cylinders, an inner cylinder that is stationary and provides for gas exchange and an outer cylinder that rotates (Fig. 2). The space between the two cylinders is completely filled with culture media and cell containing scaffolds are placed freely moving in this space. The free movement of the scaffolds leads to a microgravity environment whereas the flow of the fluid caused by the centrifugal forces of the cylinder balance with the force of gravity [40,41]. Using rat osteoblast cells, rotating wall bioreactor culture was shown to cause an upregulation in ALP, OC, and osteopontin (OPN) but no increase in cell proliferation [25]. Other studies have showed rotating wall bioreactors to be relatively ineffective for the culture of osteoblastic cells. Using rat BMSCs seeded onto poly (lactic-co-glycolic) acid (PLGA) foam scaffolds ALP and OC activity were shown to be higher in a spinner flask and a perfusion system as compared to the rotating wall bioreactor, which was not shown to be significantly different from a static control [45]. It is speculated that this rather disappointing result could be attributed to the scaffolds moving haphazardly in the system and colliding with the wall of the bioreactor. This effect was mitigated in a rotating wall bioreactor system utilizing poly (lactic-co-glycolic acid) scaffolds that are less dense than water. These scaffolds avoid collisions with the bioreactor wall and thus expressed higher amounts of ALP and calcium than scaffolds cultured in static culture [46–48]. A slight variation of the rotating wall bioreactor is used in the rotational oxygen-permeable bioreactor system (ROBS) where constructs are cultured in a 50-mL polypropylene centrifuge tube modified with a silicone elastomer to provide for gas exchange [49]. The tubes containing the constructs are then placed on a roller device and housed in an incubator. This system provides both for gas exchange and rotational shear forces and was successfully used to culture BMSCs on polycaprolactone scaffolds [12,50]. Mineralization of type I collagen was observed in scaffolds cultured in this system after 4 weeks. Using another slight variation of the traditional rotating wall bioreactor in which scaffolds are fixed to the vessel wall rather than allowed to move freely, rat osteoblasts were shown to proliferate at a greater rate and produce more extracellular matrix (ECM) proteins and mineralization as shown by alizarin red and Von Kossa staining compared to both spinner flasks and static culture [51]. Other studies comparing rotating wall bioreactors to static and spinner flask culture have been completed and have found less encouraging results for rotating wall bioreactors. Osteocalcin and ALP expression of rat marrow stromal cells has been shown to be lower in rotating wall bioreactor culture than spinner flask and static culture [41]. Similar results were also observed in a rotating wall bioreactor using hMSCs [24]. This low expression of osteoblastic markers in rotating wall bioreactors could be caused by collisions of scaffolds in the bioreactor or the low shear stresses on cells in the bioreactor. Rotating wall bioreactors are another relatively simple system that have shown effectiveness in some instances; however, perfusion systems have been shown to have greater positive effects on osteoblastic differentiation.

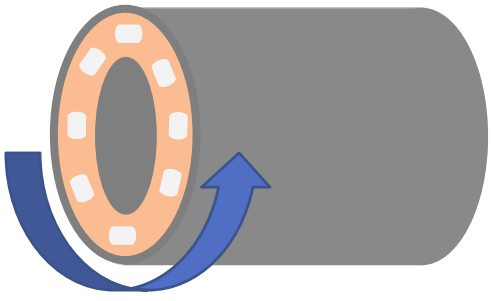

Fig. 2. Schematic of rotating wall bioreactor. Outside wall of bioreactor rotates to circulate media.

Perfusion bioreactors

Spinner flasks and rotating wall bioreactors do not effectively perfuse media into a scaffold. Bioreactors that use a pump system to perfuse media directly through a scaffold are known as perfusion bioreactors (see Table 3 for a summary of perfusion bioreactor studies). Many different perfusion bioreactor systems have been developed but most systems consist of a similar basic design consisting of a media reservoir, a pump, a tubing circuit, and a perfusion cartridge (Fig. 3) [32]. The perfusion cartridge houses the scaffold which is sealed so that media cannot flow around it, thus perfusing media directly through the pores of the scaffold. This direct perfusion makes these systems difficult to develop as the perfusion cartridge must be custom made to tightly fit a scaffold and the scaffold must have highly interconnected pores. Despite these difficulties, many perfusion bioreactor systems have been developed and tested for bone tissue engineering purposes [10,13,19,27,28,30,52–55].

Perhaps most prevalent in the literature is the flow perfusion culture bioreactor utilizing two media reservoirs to allow for complete media changes and a cassette that contains a scaffold press fit between two O-rings [27,56]. This bioreactor design has been used with an array of scaffold materials including titanium, starch-based scaffolds, and calcium phosphate ceramics [14,15,28,29,57–59]. In a study utilizing this perfusion bioreactor and rat marrow stromal cells, a continuous flow rate of 0.3–3.0 mL/min was shown to increase both the calcium matrix deposition and the rate of late osteoblastic differentiation [14]. This study utilizes a titanium fiber mesh scaffold and though little changes were seen in early osteoblastic marker ALP the effect on late osteoblastic differentiation was very pronounced. Osteopontin was measured as a marker of late osteoblastic differentiation. Based on peaks in osteopontin expression, it was concluded that fluid flow increased the rate at which the cells were differentiating. Most notable was a dramatic increase in calcium deposition in response to flow. As calcium is deposited only in late stages of osteoblastic differentiation, this result showed that the bioreactor culture was greatly enhancing differentiation of stem cells into mature osteoblasts and matrix deposition. Shear stresses in this study were reported not to exceed 1 dyn/cm^2, but exposure to these shears was attributed as the cause for the acceleration of late osteoblastic differentiation. The use of titanium fiber meshes was likely dictated by the need for highly porous scaffolds so that media can be perfused through the scaffold.

To increase the clinical relevance, a biodegradable starch-based scaffold was then used in this perfusion system [28,60]. Biodegradable scaffolds ideally degrade as new bone is being generated, so that the bone defect can heal leaving no scaffold. Nonbiodegradable scaffolds like titanium would remain even after the defect has healed. These scaffolds were shown to support osteoblastic differentiation and calcium production was shown to increase with flow. In addition, scaffolds with porosities of 75% had greater calcium deposition than those of 50% porosity. This demonstrated the combined effects of scaffold design parameters and flow perfusion. Studies evaluating the combined effects of these parameters are necessary to fully optimize a system as scaffold parameters have also been shown to influence osteogenic signal expression [61,62]. The increased extracellular matrix production observed in this study may have an effect on osteoblastic differentiation itself as demonstrated in another perfusion bioreactor study [15]. Rat marrow stromal cells were cultured for 12 days in flow perfusion cultures on titanium scaffolds and the cells removed leaving the extracellular matrix deposits. The marrow

Table 3
Studies utilizing perfusion bioreactors for bone tissue engineering grouped by bioreactor design.

Bioreactor design	Principal findings	Reference
Flow perfusion culture bioreactor	Fluid flow increases matrix deposition and accelerates osteoblastic differentiation of rat BMSCs	[14]
	Presence of extracellular matrix on titanium scaffolds enhances osteoblastic differentiation with shear stress	[15]
	Osteoblastic differentiation of rat BMSCs grown on calcium phosphate ceramics enhanced	[58]
	Flow enhances calcium deposition, porosity of starch scaffolds affects ALP expression and proliferation of rat BMSCs	[28,57]
	Rat BMSCs can differentiate into osteoblasts under flow perfusion without osteogenic supplements	[29]
	Flow enhances calcium production of rat BMSCs cultured on PLLA nonwoven meshes and improves cell homogeneity	[19]
	Flow perfusion enhances production and localization of osteoblastic growth factors TGF-β1, VEGF, BMP-2, and FGF-2	[63]
	Rat BMSC seeding improved using bioreactor system set to oscillatory flow	[7,8]
	Increasing shear stress while keeping flow rate constant increases mineralized matrix production in rat BMSCs	[18]
	hMSCs increase expression of BMP-2, BSP-2, RUNX2, OPN, and ALP in response to fluid flow	[59]
	Rat BMSC/titanium constructs cultured in bioreactor implanted in cranial defect model	[78]
Radial channel perfusion system	Improved cell number, distribution, and amounts of bone proteins with increased flow velocity of hMSCs cultured on bone plugs	[10]
	Improved cell distribution and bone matrix of hASCs in perfusion system	[70]
Complex geometry perfusion system	hMSCs cultured on decellularized bone scaffold manufactured to anatomical shape in bioreactor	[16]
Central tunnel perfused scaffold	Sheep MSCs seeded on β-tricalcium phosphate increase glucose consumption and proliferate throughout the scaffold under perfusion	[82]
	Shear stress shown to accelerate osteoblastic differentiation of human BMSCs while mass transport shown to increase differentiation among lower ranges	[83]
Direct perfusion bioreactor	Homogenous cell growth of goat BMSCs, ability to measure oxygen consumption	[75]
	Dynamically cultured goat BMSCs grown on calcium phosphate ceramic produced bone when implanted into mice	[30]
	Human BMSCs formed *in vivo* bone in mice after dynamic culture. Limited differences in osteogenic markers between dynamic and static	[17]
Tissue culture under perfusion	Human BMSCs cultured in bioreactor form bone when implanted into mice	[77]
U-tube cell seeding perfusion bioreactor system	Bioreactor used for cell seeding, enhanced number of viable seeded cells and cell uniformity	[9]
	Human BMSCs seeded directly on 3D scaffolds and cultured in bioreactor, produced bone tissue when implanted into mice	[76]
Stainless steel perfusion block	Osteoblasts seeded on trabecular bone scaffolds showed higher proliferation as lower flow rates while Runx2, osteocalcin and ALP increased with flow rate	[52]
Axial perfusion bioreactor system	Rate of mineralized matrix production of rat BMSCs increased, measured by micro-CT imaging	[84]
Gradient container	hMSCs remain viable for 14 days of *in vitro* culture and 12 weeks of *in vivo* culture	[97]
	Perfusion system increased central oxygen concentration and prevented cell death	[21]
Perfusion bioreactor system	Flow enhances proliferation and differentiation of hTERT–hMSCs	[13]
	After 40 days, higher cell densities of hMSCs in bioreactor	[86]
	Higher metabolic rates and more even distribution of hMSCs in perfusion culture, mathematical modeling completed	[87]
	Shear stress upregulated osteoblastic differentiation of hMSCs	[31]
	Dynamic culture affected ability of hMSCs to form organized matrix and altered nuclear morphology, but increased expression of OPN	[85]
	BMP-2 expression of rat MSCs impregnated with plasmid DNA encoding for BMP-2 enhanced with perfusion culture	[54]
	In vivo ectopic bone formation enhanced after rat MSCs impregnated with BMP-2 cultured under perfusion	[55]
	Significant increase in ALP and OC expression at sites of implanted collagen–PGA scaffolds seeded with rat MSCs cultured in perfusion system versus static in ectopic rat model	[53]
	Bone marrow-derived osteoblasts exhibit higher ALP and OC expression in perfusion container than static culture. Enhancement of *in vivo* bone formation was also observed in mouse skin fold	[96]
	ALP expression increases in rat osteoblastic cells cultured in perfusion container	[96]

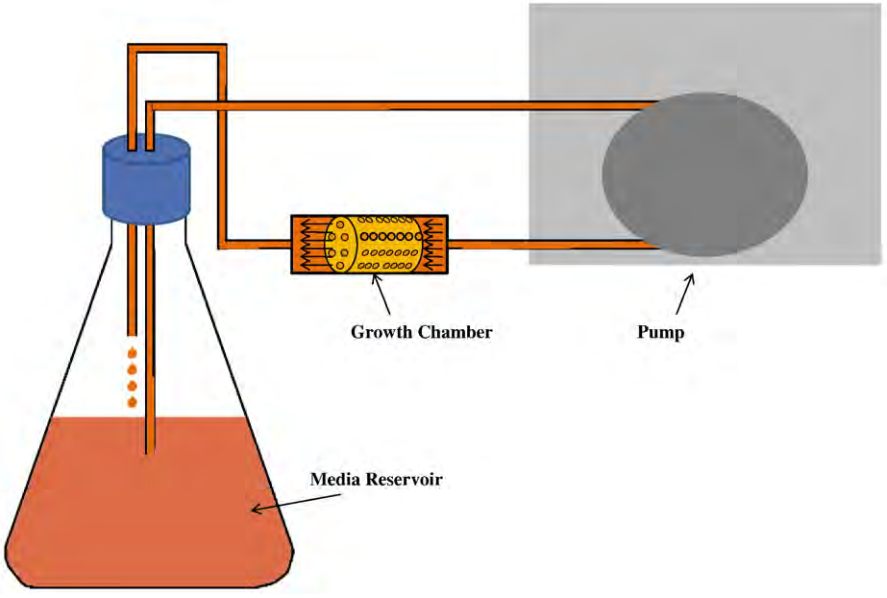

Fig. 3. Schematic of perfusion bioreactor. Media is directly perfused through porous scaffold sealed into a growth chamber.

stromal cells were then cultured in the perfusion system on the scaffolds with ECM and compared to normal titanium scaffolds. The constructs with ECM showed a 40-fold increase in calcium deposition when compared to normal titanium scaffolds even when cultured without dexamethasone, a glucocorticoid steroid widely used to induce osteogenic differentiation. This result reveals a synergistic effect between the extracellular matrix and fluid shear stress, revealing that both have a strong positive effect on matrix production. Findings of fluid shear having similar effects of dexamethasone were also reported in another study using this bioreactor system [29]. Samples cultured for 16 days showed significant increases of calcium deposition under flow perfusion without dexamethasone as compared to static cultured samples with dexamethasone. Adding dexamethasone to the flow culture further increased calcium production. Osteopontin levels in bioreactor groups cultured with dexamethasone were increased to levels greater than static culture with dexamethasone and bioreactor culture without dexamethasone indicating that flow has a synergistic induction effect.

The role of growth factors in bioreactor culture should also be investigated to fully elucidate the effect of perfusion systems on osteoblastic differentiation. Dynamic culture not only could enhance the production of growth factors through cell stimulation but also potentially reduce the local concentration of soluble factors through increased mass transport. One such study utilized polycaprolactone scaffolds and analyzed the localization of several endogenously expressed growth factors including transforming growth factor beta 1 (TGF-β1), fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), and bone morphogenetic protein-2 (BMP-2) [63]. Using immunohistochemistry, positively stained areas increase with increasing flow rate indicating flow enhanced expression of these growth factors. *In vitro* enhancement of these growth factors could enhance *in vivo* bone growth. For example, VEGF, an angiogenic growth factor, has been used to enhance endothelial cell proliferation and encourage vessel sprouting aiding in the vascularization of an implanted construct [64,65]. BMP-2 has been widely investigated for its role in enhancing *in vivo* bone growth and *in vitro* osteoblastic signaling [66–69]. *In vivo* studies should be performed to establish that the increased *in vitro* production of these growth factors translates to enhanced *in vivo* bone formation.

Shear stress has been shown to play a substantial role in the increased matrix production observed in perfusion culture, but it is also possible that the increased media flow could also cause this result. The contribution of nutrient availability and shear stress have been examined independently in studies which use a thickening agent to increase media viscosity and are discussed later in this review. In 9 mm in diameter and 5 mm in height scaffolds seeded with a preosteoblast line, central oxygen concentration dropped to 0% in 5 days of culture [21]. Cell death was subsequently observed in these areas of the scaffold. When the demineralized bone matrix scaffolds were placed in a perfusion cartridge, central oxygen concentration was raised to 4%. Although this oxygen concentration is still lower than the bulk media (~20%), it was high enough to prevent cell death. Even in relatively small scaffolds (cells in these scaffolds were no more than 2.5 mm from the scaffold surface), concentration gradients occur in static culture, underscoring the importance of systems that enhance nutrient transport to cells.

The ultimate goal of these perfusion systems is to develop a method to grow cells *in vitro* that can be implanted to repair bone injury sites. Using a perfusion design made of a custom machined piece of polycarbonate with space for up to six scaffolds, hMSCs were shown respond to shear, increasing amounts of deposited protein [10]. This same culture system was shown to be effective for the osteoblastic differentiation of human adipose-derived stem cells (hASCs) [70]. hASCs have been used as an accessible cell source for osteoblasts as an alternative to bone marrow derived MSCs [71–74]. The distribution of cells and bone matrix in the scaffolds was shown to increase in the bioreactor system compared to static culture. Increasing the clinical relevance of the work, the same bioreactor system was used to culture a demineralized bone scaffold machined to a defect shape based on CT scans. When hMSCs were cultured on the construct, cell number and bone volume were both significantly greater in the bioreactor as compared to a static control [16]. This study revealed a strategy of how bioreactors could be used to aid in the regeneration of bone tissue, but limited *in vivo* studies have been completed to assist in bringing such a strategy to the clinic.

In one such *in vivo* study, calcium phosphate ceramics seeded with goat bone marrow stromal cells were cultured in a perfusion bioreactor and implanted subcutaneously in mice [30]. The scaffold system could be used to produce as much as 10 cm^3 of bone like engineered tissue, but the sample size of the study was small and statistical difference was not observed between bioreactor and static culture. The perfusion system used consisted of a growth chamber, pump, oxygenator, and media reservoir. Oxygen probes were added before and after the growth chamber, and measurements from these probes were used to predict cell doubling time based on oxygen consumption [75]. Human stem cells were then cultured in the bioreactor system, with the goal of demonstrating bone tissue growth, and although bone growth was observed after implantation in nude mice, no significant differences in bone formation or osteogenic signaling was observed between dynamically and statically cultured constructs [17]. While this result demonstrates the efficacy of using bioreactor systems, these systems must be demonstrated to enhance bone formation if bioreactor systems are to be utilized in a tissue engineering strategy. In addition to illustrate clinical relevance, this result should be demonstrated in a bone defect model. In a different study again using a skin fold model, human BMSCs were seeded on hydroxyapatite scaffolds and grown in a bioreactor also used for cell seeding [9,76]. Increased bone formation was observed when the constructs were implanted in an ectopic mouse model as compared to static controls. Quantitative scoring of hematoxylin and eosin staining was used to gain these results, but nonetheless demonstrate the efficacy of using bioreactor systems; however, more studies are necessary to confirm this result. Small sample sizes and large errors could make it difficult to observe significance in *in vivo* models, but careful experimentation must be completed to conclude whether or not bioreactor systems can improve *in vivo* bone growth. Another advantage of *in vivo* models is a further demonstration of clinical relevance. In one study, osteoconductive grafts were created using a different seeding method that could increase clinical relevance [77]. Rather than isolate BMSCs from bone marrow by growing the cells in monolayer, whole bone marrow was isolated, bone marrow stromal cells were enriched using a density gradient, and these cells were seeded directly into the bioreactor system, rather than first being expanded in 2D. Bone tissue formation was observed in a mouse ectopic model using these constructs. Potentially increasing the clinical relevance of bioreactor work, this result is interesting as adding BMSCs directly to scaffolds without prior expansion would shorten the time and difficulty required in implementing a tissue engineering strategy. These *in vivo* experiments were completed in a skin fold model, but *in vivo* studies using a bone defect model have also been completed.

Using the flow perfusion culture bioreactor, rat BMSCs were cultured dynamically on titanium scaffolds and implanted in a rat cranial defect model [78]. After 30 days, bone growth was observed in all cell groups, but there were no significant differences between the treatment groups of perfusion versus static and varying *in vitro* culture times. The authors speculate that this could potentially be again attributed to the difficultly of observing statistical significance in *in vivo* models. This study demonstrates the principle of a bone tissue engineering strategy using a perfusion bioreactor. Further *in vivo* studies utilizing perfusion bioreactors are required to prove the effectiveness of these systems especially those that use a bone defect

model rather than simply implanting the construct subcutaneously. The lack of *in vivo* studies could potentially be explained from the difficulty of developing and testing a perfusion system. Because of the need for custom-made parts and the difficulty of perfusing media directly through a scaffold development and maintenance of these systems can be time-consuming. These shortcomings need to be overcome, and an increased amount of *in vivo* studies need to be completed to demonstrate the clinical relevance of perfusion bioreactor systems. The clinical relevance of perfusion bioreactor systems will be defined by their ability to be used with other strategies for bone tissue engineering including biodegradable scaffolds, scaffolds and growth factors that enhance osteoinduction and osteoconduction, and readily available stem cell populations. Much of this work has begun to be completed; however, additional studies focusing on scaffold parameters such as pore size and stiffness and exogenous growth factor delivery should be completed in conjunction with bioreactor studies to observe how bioreactors influence cell response to these factors. The ultimate advantage of bioreactor systems over conventional techniques is the ability of bioreactor systems to produce reproducible culture conditions that enhance stem cell proliferation and differentiation and can be made to minimize handling of scaffold and human labor. By automating the process of 3D cell culture, a bone tissue engineering strategy can be developed that can be feasibly implemented in the clinic on a large scale.

Effect of shear stress and mass transfer on proliferation and osteoblastic differentiation

Studies focused on the effects of fluid shear on osteoblastic differentiation have shown that shear stress affects osteogenic signal expression of mesenchymal stem cells [36,37–39,79,80]. Studies reported in this section utilize laminar flow regimes. In addition to the velocity of flow which affects the magnitude of shear, studies utilize flow patterns including oscillatory and continuous flow. Nearly all long-term three-dimensional bioreactor studies utilize a continuous flow rate, while many short two-dimensional studies analyze continuous, pulsatile, and oscillatory flow. Because most long-term three-dimensional bioreactor studies use continuous flow regimes, this section focuses on 2D and 3D flow systems that utilize continuous flow. In one such study utilizing a flow that occurred for 5, 30, or 120 minutes every other day for 20 days, shear stresses of 1.6 dyn/cm^2 were used, and osteopontin and bone sialoprotein (BSP) expression were shown to increase in response to shear [38]. Significant changes in osteogenic signal expression have also been observed after shorter exposure to shear stresses in this range [39]. When rat BMSCs were exposed in 2D to shear stresses of 2.6 dyn/cm^2, significant changes in osteogenic signaling pathways were observed. Shear stress was administered for periods of 30 minutes to 24 hours, and flow regimes were either continuous or intermittent. After 30 minutes, flow was shown to significantly enhance the phosphorylation of mitogen-activated protein kinases (MAPK), p38, and extracellular signal-related kinase (ERK). To test if the effect of shear stress on MAPK signaling processes also influenced additional osteogenic signal expression, collagen 1α1, osteopontin, cyclooxygenase-2 (COX-2), and VEGF were analyzed. No changes after 4 hours of flow were observed in osteopontin or collagen 1α1, but COX-2 and VEGF were significantly upregulated. However, when cells were exposed to shear for 24 hours and then cultured in static conditions for 14 additional days, significant upregulation of osteopontin, collagen 1α1, bone sialoprotein, and osteocalcin were observed, indicating that this early impact on osteoblastic signal expression affected late osteoblastic differentiation [39]. This effect on early osteoblastic differentiation was observed at a high shear stress when human osteoblast ALP protein levels were shown to double after just 30 minutes of exposure to 20 dyn/cm^2 shear stresses [37].

Long-term exposure to shear stresses an order of magnitude lower has been shown to generate similar results [79]. Human bone marrow stromal cells were exposed to shear stresses of 0.012 dyn/cm^2 for 10 days using a two-dimensional parallel plate. Following exposure to the shear stress, the cells were shown to proliferate at a slower rate as compared to static culture; however, immunohistochemical staining for type I collagen and Von Kossa staining showed more intense staining for fluid flow groups. This indicates that cells exposed to shear are producing more calcium and collagen, components of bone extracellular matrix. Signal expression data showed mixed results after exposure to shear with upregulation of collagen type I and osterix, but downregulation of ALP and OC. BSP and OPN were also analyzed, and these genes were either upregulated or downregulated by shear depending on substrate type [79]. These studies on short- or long-term shear rate in 2D cultures indicate that osteogenic signal expression is greatly affected by fluid shear stresses with magnitudes as little as 0.01 dyn/cm^2 and as high as 20 dyn/cm^2. These 2D studies allow for greater control of experimental variables and can give beneficial information on how MSCs and osteoblasts are responding to shear.

Bioreactor studies using three-dimensional scaffolds also provide information about shear, but additional variables in the systems can make it difficult to calculate exact shear stresses cells are exposed to. Complex modeling is required to accurately compute flow rate induced shears from fluid flowing through the pores of three-dimensional scaffolds, and these shears are influenced by factors that are difficult to measure including cell growth and extracellular matrix deposition. These two factors can effectively alter the path that fluid flows, thus altering local velocities and shear rates. Bioreactor systems also enhance mass transport, which could also be influencing osteogenic signal expression, as well as influencing local concentrations of soluble factors influencing osteoblastic differentiation. Long-term perfusion bioreactor studies have reported shear stresses from 0.05 to 1.0 dyn/cm^2 (Table 4). Increased calcium matrix production is often observed in these studies, and this outcome is often attributed to shear stress. Based on short-term and 2D studies, this is a valid conclusion, and it is further verified by studies that isolate the effects of shear stress by holding mass transport constant and increasing shear through the use of thickening agents such as dextran. The addition of dextran to culture media increases the viscosity and dextran itself does not affect osteoblastic differentiation [81]. Two studies that evaluate the direct effect of shear stress on osteoblastic differentiation in three-dimensional perfusion systems are reported.

One of these studies performed in a bioreactor system previously discussed and utilizing titanium fiber mesh scaffold used dextran to increase fluid viscosity 2- and 3-fold [18]. As viscosity increased, calcium deposition also increased, with a 7-fold increase as a result of the 3-fold viscosity increase. Although the shear stresses in the system were still relatively low, the extended culture time (16 days) with fluid flow induces the changes in osteoblastic differentiation. Shear is an important variable to isolate in bioreactor systems as most studies that increase shear also increase flow rate, enhancing mass transport. Isolating the effect of shear from mass transport enables researchers to greater understand the results of their experiments. The effect of this increased mass transport from perfusion systems was studied in detail in a recent work. This study uses a perfusion system in which a porous β-tricalcium phosphate scaffold (TCP) is placed in a media reservoir and a tube sealed into an opening in the middle of it. Media is then pumped from the reservoir by a pump into the opening and the media is forced through the pores perfusing the scaffold [82]. Flow rate was first held constant while the shear stress increased through addition of dextran to the media. In other experimental groups, the shear stress was held constant while the flow rate was increased, permitting the effects of both shear stress and mass transport to be analyzed separately. Results revealed that increasing mass transport increases osteogenic markers ALP and OPN over lower values (0–

Table 4
Comparison of shear stresses reported in bioreactor studies.

Reported shear stress (dyn/cm^2)	Effects on osteoblastic differentiation	Reference
Not exceeding 1.0	Significant acceleration of late osteoblastic differentiation (calcium deposition and osteopontin) in rat MSCs throughout 16 days of culture	[14]
0.2–0.3	Significant increase in calcium deposition in 0.3 dyn/cm^2 compared to 0.2 dyn/cm^2 (porosity changes from 75% to 50%) over 15 days in rat MSCs	[57]
0.05	Greater calcium deposition after 16 days of culture of rat MSCs	[19]
0.1–0.3	Increasing shear stress by increasing viscosity 2× and 3× increased calcium deposition of rat MSCs 4 and 7 fold after 16 days	[18]
0.1	Using hASCs perfusion culture increased expression of collagen, bone sialoprotein and osteopontin compared to static	[70]
0.007–0.1	Increasing perfusion rate increased cell number distribution and protein production of hMSCs over 5 weeks	[10]
<0.05	Lower calcium content as compared to spinner flask using hMSCs	[22]
1.6	BMSCs cultured for twenty days exposed to flow every other day for 5, 30, or 120 minutes showed greatest expression of late osteoblastic markers after 30 min	[38]
0.05–0.15	Holding flow rate constant while raising shear from 0.05 to 0.15 dyn/cm^2 increased mineralization and accelerated osteoblastic differentiation of human BMSCs	[83]

6 mL/min), but begins to have an inhibitory effect at 9 mL/min [83]. This likely occurs as a certain flow rate is required to sufficiently supply nutrients to the cells, but once that flow rate is reached, high mass transport rates interfere with cell–cell signaling mechanisms. This result indicates that there is an optimal mass transport rate that exists. As in previous studies, increased shear stress (approximately 0.11 dyn/cm^2 to 0.15 dyn/cm^2) induced a higher amount of OPN and OC activity at 28 days. It is also likely that a maximum shear stress exists above which either no further enhancement of cell signaling is observed or that shear begins to be inhibitory. This study, however, did not reach that level. The result that shear stress can accelerate osteoblastic differentiation is confirmed by this study, but it also reveals that this effect occurs in tandem with increased mass transport.

Mathematical modeling can be an important tool to determine what shear stresses a bioreactor system exposes cells to and how the system affects mass transport. These analyses can be difficult to complete as flow through random porous architecture scaffolds can be difficult to model and this architecture changes with cell growth. A possible way to account for cell growth would be to combine mathematical modeling with an advanced imaging technique that could map cell growth and matrix production [84]. A mathematical model that does not account for cell proliferation was completed on a system shown to increase stem cell osteoblastic differentiation and growth [31,85–87]. According to the simulation, cells in the scaffold experienced shear stresses from 0.0001 to 0.001 dyn/cm^2. These shears are low compared to others reported in the literature and were shown to only penetrate 70 μm into a 1.2-mm-diameter scaffold. Analysis of oxygen content revealed that oxygen levels were sufficient for cell growth, so any differences in cell behavior observed could be attributed to the shear stresses in the system. Mathematical models of bioreactor systems such as this one should be completed on bioreactor systems to add insight into the mechanisms behind cell behavior in the systems. Shear has been shown to influence osteoblastic differentiation, but it is unknown exactly what magnitude of shear stress cells are exposed to in many bioreactor systems. Modeling should be used as a tool to determine these values and yield information to explain the phenomena behind increased stem cell growth and osteoblastic differentiation. Detailed math models, experiments isolating shear stress and mass transport, and monitoring of oxygen and nutrient concentrations are all tools to determine what effect bioreactor shear stress and mass transport have on stem cells. Short-term experiments exposing osteoblasts or stem cells to shear stress in monolayer have provided insight on the cell signaling pathways affected by shear stress, but analysis in perfusion systems must also be completed to determine the effect of long-term shear stress. Determining this effect will allow for a greater understanding of perfusion systems and lead to more effective optimization of these systems and potentially a more effective introduction of these systems into clinical bone tissue engineering.

Bioreactors for cell seeding

This review has mainly focused on bioreactor systems for the long-term culture of bone tissue engineering constructs; however, the use of bioreactors for cell seeding represents another important use of these systems. Cells seeded on tissue engineering constructs are often loaded by directly adding a cell suspension to the scaffold. Although this method is simple and thus widely used, it can result in low seeding efficiencies and nonhomogenous seeding distributions [7,9,88]. Seeding efficiency can be improved by placing porous scaffolds in a mixing cell solution. Spinner flask systems have been used for this purpose; however, perfusion bioreactor seeding systems have shown to further improve seeding efficiency [7–9,20,86,88–91]. Bone marrow stromal cell and chondrocyte loading efficiency have been shown to be 20% higher using a perfusion system as compared to static and spinner flask loading and significantly more uniformly distributed [9]. Using another bioreactor system that has been discussed in this review, the flow perfusion culture bioreactor, oscillatory flow was used to seed preosteoblastic cells on polystyrene matrices and foams as well as PLLA scaffolds [7]. Dynamic seeding yielded higher seeding efficiencies under most culture conditions. Even more relevant to bioreactor bone tissue engineering, perfusion seeding yielded higher cell attachment after exposure to shear when compared to statically loaded cells exposed to shear. This has important ramifications for bioreactor systems as most perfusion systems are statically seeded and then loaded into a bioreactor. Perfusion seeding may increase seeding efficiency and reduce risk of contamination by decreasing the amount of time the scaffolds are handled outside the bioreactor system. Further studies indicate that flow rate used in dynamic seeding of PLLA scaffolds influences scaffold cellularity with lower flow rates yielded higher cellularity [8]. The exact nature of this relationship will likely vary with bioreactor type, scaffold material and porosity, and cell loading concentration, thus it should be customized for each seeding perfusion bioreactor. The use of perfusion bioreactors for cell seeding represents another aspect of bone tissue engineering that bioreactor systems can improve upon. Continued experimentation in this area could lead to a bone tissue engineering strategy in which cells are loaded onto scaffolds in bioreactor systems and cultured long-term in the same system, maximizing efficiency and minimizing contamination risk.

Commercial bioreactor systems

Despite the hurdles to developing a bioreactor system for bone tissue engineering some bioreactor systems are currently on the market or in the process of commercial development. Several of these systems are summarized in Table 5. Some of the products listed in Table 5 are not specifically designed for bone tissue engineering; however, these systems could potentially be used for a bone application. Others have been used for bone tissue engineering and

Table 5
List of companies with commercial bioreactor systems.

Company	Product description	Web site
Aastrom	Developing bioreactor system for stem cell expansion	www.aastrom.com
Histogenics	Developing NeoCart® autologous engineered neocartilage which utilizes bioreactor system	www.histogenics.com
New Brunswick	Bioreactor systems for scale up of mammalian cells	www.nbsc.com
Minucell and Minutissue	Various bioreactor systems for 3D tissue culture including gradient container, container tissue factory, and perfusion culture container	www.minucells.de
Synthecon	Produce many batch systems including the NASA developed Rotating Cell Culture System and a Perfused Culture System	www.synthecon.com
Pluristem Therapeutics	Patented PluriX™ 3D Bioreactor for expansion of marrow stromal cells	www.pluristem.com
FiberCell™ Systems Inc.	Manufacture Hollow Fiber bioreactors that can be used for endothelial cell and other mammalian cell culture	www.fibercellsystems.com
Biovest International	AutovaxID™ automated cell culture system for use in mammalian cell culture	www.biovest.com
Wyle Labs and Celdyne	Hydrodynamic focusing bioreactor developed by NASA for cell expansion and culture	www.wyle.com

have been reported in the literature including the gradient container, and perfusion containers [92–97]. Following culture of BMSCs seeded on β-TCP scaffolds in the Minucell and Minutissue perfusion container system, a higher expression of ALP was observed throughout a 4-week study as compared to a static control [95]. Osteocalcin was also shown to increase in this system as well as *in vivo* subcutaneous bone growth [96]. Results from studies utilizing rotating wall bioreactors marketed by Synthecon have also been published in the literature [25,98,99]. Human BMSCs seeded on porous silk scaffolds were shown to produce homogenous bone like constructs after 5 weeks of culture in the Synthecon rotating wall bioreactor [99]. Studies like these completed using commercially available systems are highly valuable as these studies have increased clinical relevance as commercial systems can be purchased by multiple labs and have already begun to be mass produced. Development, testing, and experimentation on commercial systems should be continued as currently these systems are not approved by the FDA for clinical use. Companies motivated by commercial use will have incentive to seek regulatory approval on bioreactor systems. Thus bioreactor systems can be developed beyond what is done in academic labs.

Conclusions and future directions

A significant volume of work has been reported to support the use of bioreactor systems for bone tissue engineering. Spinner flasks and rotating wall bioreactors can be readily implemented for culture of three-dimensional constructs, and these systems have shown some promising results, but the inability of these systems to greatly enhance nutrient transfer throughout a scaffold limits the degree of improvement over static culture. However, use of these systems should be continued at least into the near future as implementation of these systems is more readily achieved than more complicated perfusion systems. Experiments utilizing perfusion systems have shown very promising results including induction of osteoblastic differentiation without dexamethasone [15,29] and growth of cells on a scaffold custom designed for a bone defect [16]. Despite these successes, more progress needs to be made in order for perfusion bioreactor systems to be used in a clinical setting. Researchers should focus on the clinical strategy for implementing their bioreactor system including improving ease of use, minimizing failure rates, and optimizing cell proliferation and differentiation (Fig. 4). This focus should include the entire strategy including cell type (readily available cells sources such as MSCs should be focused on), biomaterial (nontoxic biodegradable scaffolds), and bioreactor components (FDA approved). Attention to these items will allow for more rapid transference of bioreactor systems to a clinical setting. Furthermore, research should be conducted in a linear manner, with experiments focusing on demonstrating effectiveness in a clinical setting. Despite the plethora of work completed on perfusion bioreactor systems, minimal work has been reported utilizing perfusion bioreactor cultured bone tissue engineering constructs in an animal defect model. Given the promise and amount of research conducted on many bioreactor systems, a defect experiment must be the next step to utilize perfusion bioreactors in a clinical setting. Once

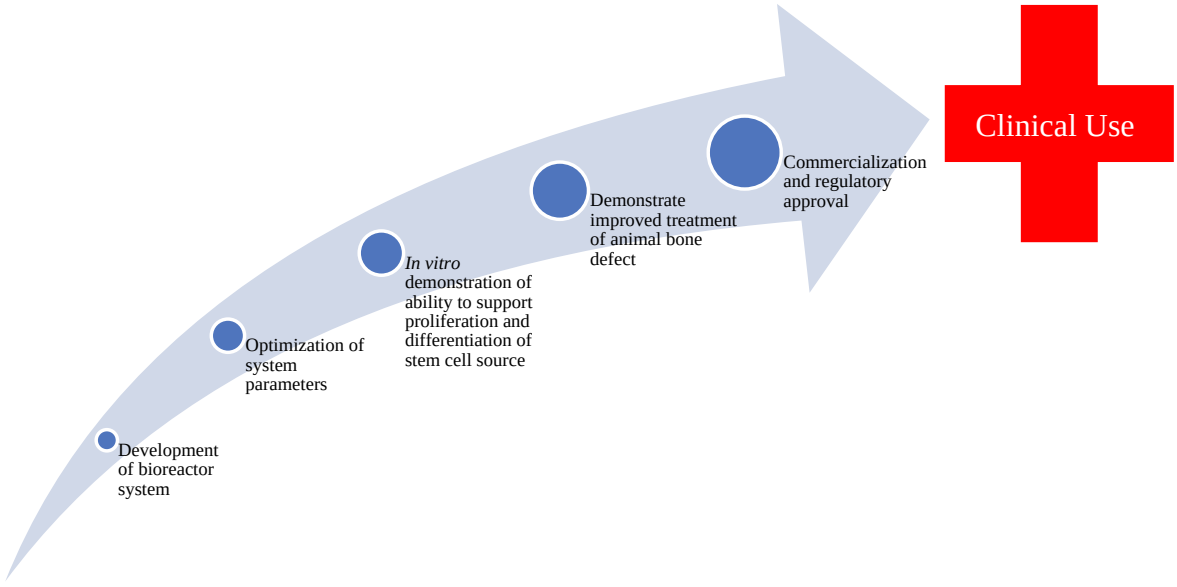

Fig. 4. Clinical roadmap for bone tissue engineering bioreactors.

these studies are completed, researchers should focus on commercializing their bioreactor system and overcoming regulatory hurdles to begin clinical use. The potential associated with these systems is great, but significant additional progress must be made. Ideally, a bioreactor-based bone tissue engineering strategy would start with the extraction of a stem cell population from a patient. This population would then be uniformly seeded on biodegradable, biocompatible three-dimensional scaffolds in a bioreactor system. The construct would be cultured within an environment that would cause the stem cells to rapidly proliferate and undergo osteoblastic differentiation with the bioreactor system monitoring oxygen content and automatically changing media when necessary. After a short but sufficient culture time, this construct would be removed and directly implanted in the patient where it would foster osteoinduction and osteoconduction for rapid repair of the defect site. The scaffold would biodegrade, leaving only regenerated bone tissue where there was once an injury. With an increased amount of relevant animal studies and development of a clear strategy, bioreactor systems could play a key role in a tissue engineering treatment for bone defects and bring this strategy to clinical reality.

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