PRODUCTION OF BIODEGRADABLE MICROSPHERE-BASED 3D POLYMER SCAFFOLDS FOR BONE TISSUE ENGINEERING

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ABSTRACT

This paper presents laboratory production of biodegradable microsphere -based scaffolds for the treatment of critical-sized fracture in bones. Whereas a common bone fracture heals up naturally when put together in a POP cast, a critical-sized fracture does not—a special bioengineering intervention is thus required to produce regeneration. A promising bone regeneration strategy is to develop 3D scaffolds that can mimic structural and compositional characteristics of the natural bone extracellular matrix (ECM). Using microsphere sintering technique, a polymer solution was prepared by dissolving poly(lactic-co-glycolic) acid, PLGA in methylene chloride. The solution was added to a 1 % poly (vinyl alcohol), PVA solution to form an emulsion which was continuously centrifuged for over 20 hours. Water-drenched nano/micro polymer spheres which were formed were isolated by vacuum filtration. After washing with deionized water, the microspheres were lyophized in liquid nitrogen for 24 hours. Light and porous 3D scaffolds were fabricated from the microspheres by thermal sintering of 500-700 µm microspheres in stainless steel mould at 70 ^{o}C for 1 hr. SEM micrographs of synthesized scaffolds attest to the structural competence of 3D scaffolds. In regenerative engineering, this microsphere technology provides a nanotechnology solution approach to the limitations projected by surgical autografts and allografts in bone tissue repair.

Keywords:
scaffold, fracture, nanotechnology, sintering, regenerative engineering, autograft, allograft, microsphere, lyophize, PLGA

1. Introduction

The skeletal system supports the body and provides protection for its vital organs. Musculoskeletal trauma mainly includes bone fractures; however, injuries of the ligament and joint, and soft tissue diseases related to muscle may also be categorized in this group. Several bone defects and nonunions caused by trauma, pathological degeneration, tumor resection, or congenital deformity have been traditionally treated by using allograft and autograft bones [1,2]. Nevertheless, the application of autografts is limited due to the donor shortage and donor-site morbidity; while application of allografts is limited by the high risk of diseases transmission and immune rejection [3-8].

Regenerative engineering approaches utilizing biomimetic synthetic scaffolds provide

alternative strategies to repair and restore damaged bone--the efficacy of the scaffolds for functional bone regeneration critically depends on their ability to induce and support vascular infiltration [9]. The bone as a natural composite requires a careful materials selection for proper replacement. With increasing environmental pollution and advances in medicine, biodegradable polymers have become a subject for research for two main applications—medical treatment and environmental waste control. Biopolymers already find extensive use in the medicine as drug delivery systems, internal fixation devices, surgical sutures and tissue engineering scaffolds [10,11]. Biodegradable polymers may be natural or synthetic. Biopolymers include cellulose, starch, and chitin, while the more common synthetic polymers consist of poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), and poly(glycolic acid) (PGA) [12]. Among these synthetic polymers, PGA and PLA have been researched together with their copolymer— poly(lactic-co-glycolic acid) (PLGA). The present study aims at synthesizing polymeric microspheres and fabricating them to 3D scaffolds for bone tissue regeneration.

2. Materials and Method

Prominent materials and equipment employed in this study are briefly highlighted below:

Materials:

1. Poly(lactic-*co*-glycolic) acid (PLGA 85:15. i.e., 85% lactic acid and 15% glycolic acid)
2. 1 % poly (vinyl alcohol), PVA solution
3. Methylene chloride
4. Deionized distilled water
5. Liquid nitrogen

Equipment:

1. Fume hood
2. Centrifuge
3. Precision Pipette
4. Magnetic/mechanical stirrer
5. Vacuum filter
6. Micron sieve
7. Stainless steel mould
8. Oven

The synthesis is basically simple. A polymer solution was prepared by dissolving PLGA in ethylene chloride (ratio1 :4 wt/vol). 1% polyvinyl alcohol (PVA) was prepared by adding PVA to water in the ratio 1:99 by volume. The 1% PVA solution was added to the PLGA polymer solution at room temperature to form an emulsion. The resulting polymer emulsion was continuously stirred mechanically for 24 hrs. A cluster of nano/micro polymer spheres was formed and was isolated by vacuum filtration. The spheres were washed with deionized water about three times and then air-dried for another 12 hrs. A test tube containing the polymer nano/microspheres was carefully lowered into a cooled flask containing liquid nitrogen. A frozen solid mass instantly engulfed the nano/microspheres and was set up in a fume hood for 24 hours. This process is termed ―lyophilizing‖ i.e., freeze-drying. It further helps to remove water from the nano/microspheres. Microspheres of size range 500—700 µm were collected using a micron sieve and sintered in a steel mould at 70^{o}C for 1 hr in an oven.

3. Results and Discussion

The experimental work resulted in production of mechanically stable 3D scaffolds as shown in Fig 1. These scaffolds are currently being researched for mechanical integrity and

stability. Already, Scanning Electron Micrographs of scaffolds have been obtained with very encouraging initial results, Fig 2. The emergence of synthetic bone repair scaffolds has been necessitated by the limitations of both autografts and allografts (i.e., shortages of supply and risk of disease transmission).

Fig 1: Mechanically stable 3D scaffolds

Why using scaffolds for bone regeneration? What has become clear over the past decade in bone repair is that cells alone are not enough for orthopedic repair—they need scaffolds or materials for physical structure (as structurally illustrated in Figs 2 & 3) to retain cell populations after implantation or repopulation, to guide multipotential cells that are implanted or delivered to the repair site, or to provide a template for cells to lay down new extracellular matrices in the bone structure.

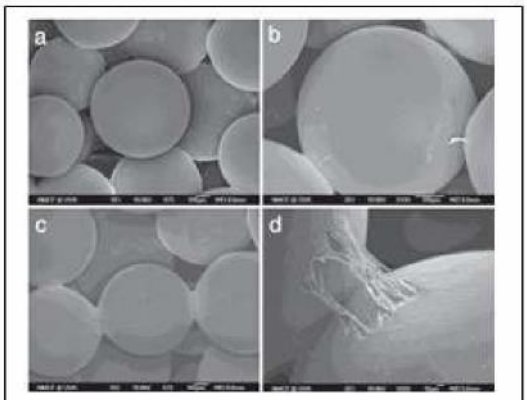

Fig 2A: Scanning electron micrographs showing adipose-derived stromal cell (ADSC) proliferation at days 7 and 14 on PLAGA sintered microsphere scaffolds. (*a*) Day 7. (Magnification: X 75.) (*b*) Day 7. (Magnification: X 150.) (*c*) Day 14. (Magnification:X75.) (*d*) Day 14. (Magnification: X 350). (Jabbarzadeh,et al, 2008)

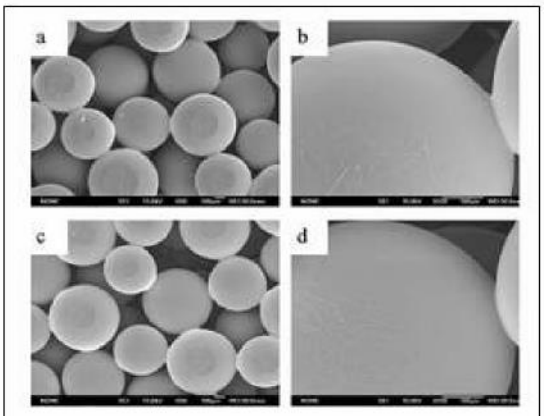

Fig 2B: Scanning electron micrographs indicating the effect of acetic acid treatment on the morphology of PLAGA scaffolds. No obvious morphological change was observed on PLAGA scaffold before (a and b) and after (c and d) acetic acid treatment. Magnification: X50 (a and c) and X 200 (b and d) (Jiang et al, 2010)

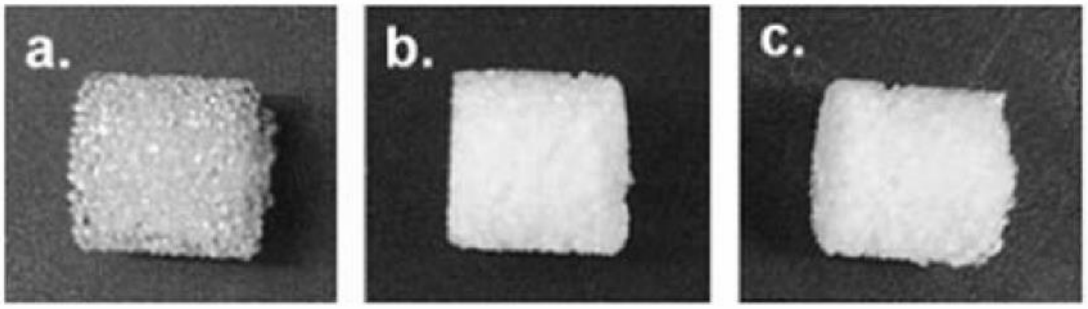

Fig 2C: Scaffold image comparing (a) PLAGA, (b) HIGH (83% PLAGA, 17% HA) and (c) LOW (73% PLAGA, 27% HA) scaffolds. PLAGA scaffolds are notably translucent, while both PLAGA– HA composite scaffolds are opaque (Emily et al, 2007)

Fig 2: Scanning Electron Micrographs of microsphere

The primary focus of the present work is to produce PLGA microspheres. Part of the objectives however, is to produce nanospheres with incorporated nanofibrous extracellular matrix (ECM). The nanospheres are being formed from appropriate combination of four variables:

1. Concentration of the PVA solution (surfactant)
2. PLGA—Methylene Chloride ratio
3. Agitation rate
4. Duration of agitation

It is expected that a nanosphere-based scaffold will serve better in bone tissue regeneration owing to the following reasons:

1. Smaller size
2. Higher packing density
3. Fine serrated surface
4. Improved porosity

Preliminary results showed that above-listed factors result in an enriched morphological surface and structure with thousands of inter-connected through pores described by the

structural framework of Fig 3 & 4.

Fig 3: Structural Scaffolding

Fig 4: Scaffolding in construction

The bone in its natural body environment is surrounded by extracellular matrix (ECM) during endogenous ossification of long bones. Mimicking this natural environment might contribute to faster regeneration rate during healing. In order to mimic natural bone ECM, we produce nanofibrous meshes in the pores spaces of a mechanically stable, porous, sintered, nanosphere matrix. Millions of channels are thus created for migration of multipotential cells to proliferate and cause regeneration as illustrated in Fig 5.

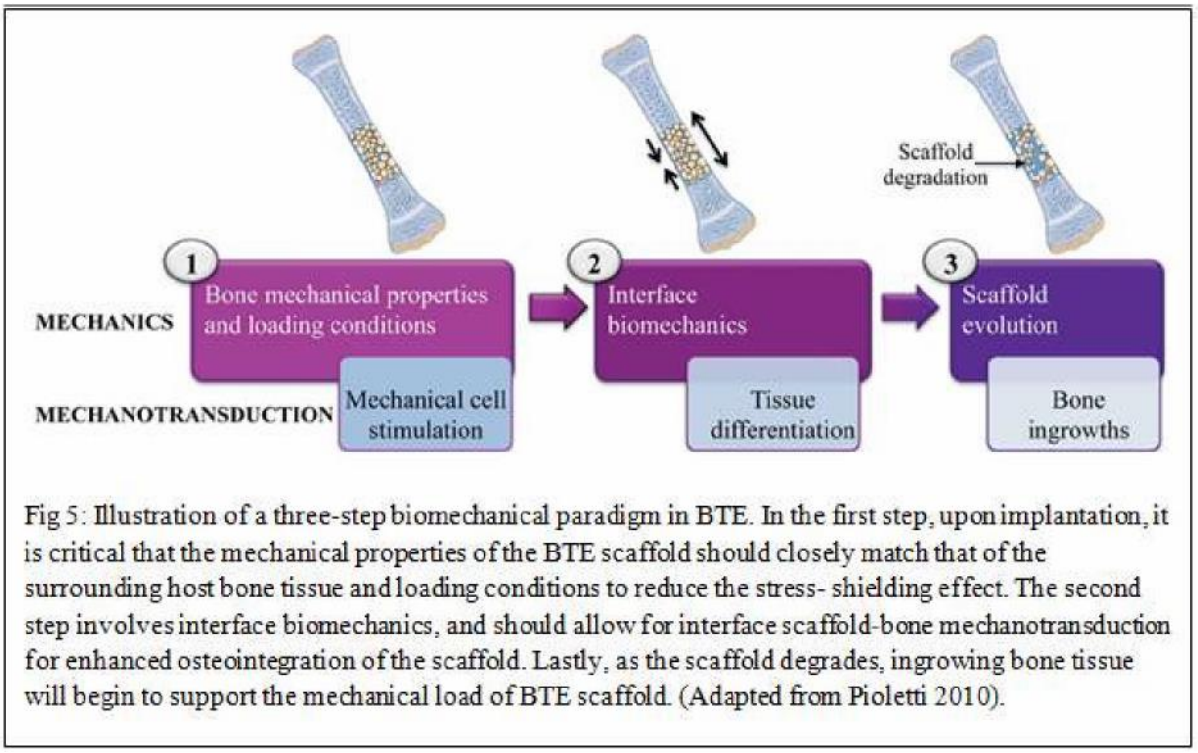

Fig 5: Illustration of a three-step biomechanical paradigm in BTE. In the first step, upon implantation, it is critical that the mechanical properties of the BTE scaffold should closely match that of the surrounding host bone tissue and loading conditions to reduce the stress- shielding effect. The second step involves interface biomechanics, and should allow for interface scaffold-bone mechanotransduction for enhanced osteointegration of the scaffold. Lastly, as the scaffold degrades, ingrowing bone tissue will begin to support the mechanical load of BTE scaffold. (Adapted from Pioletti 2010).

4 Conclusion

The following were drawn from the experiment conducted and described in this study

1. Bio-structural support (scaffolds) for critical-size fracture in bones was produced from biopolymer PLGA.
2. The scaffolds were produced from chemical synthesis and thermal sintering of PLGA polymer microspheres.
3. The synthesis was a combined formulation of microspheres and nanospheres. The microspheres were harnessed and sintered successfully to form mechanically stable 3D scaffolds.

4. Scanning Electron Micrographs obtained attests to the excellent microporous structure of the scaffold which offers a unique hope of bone cell mobility and proliferation. *Porous structure* of the scaffold is one important factor that accelerates regeneration of fractured bones.
5. Biopolymer Microsphere Scaffolds (BMS) is a promising alternative to autograft and allograft bone in combating musculoskeletal trauma.
6. The study is ongoing with both *in vitro* and *in vivo* scope in focus.

Acknowledgements

The authors wish to appreciate the following at the indicated capacities.

Project Sponsor:

World Bank Science and Technology Post Basic (STEP-B)

Technical Partners:

a. National Agency for Science and Engineering Infrastructure (NASENI), Abuja, Nigeria
b. African University of Science and Technology, (AUST) Abuja, Nigeria

Host Institution:

University of Connecticut Health Center, (UCHC), Farmington, Connecticut, United States of America (USA).

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