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The Fabrication of a Biomedical Scaffold from Human Placenta

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ABSTRACT

The powder derived from human placenta (HP) was successfully used in regenerative medicine. The purpose of this study was to fabricate powder from the human placenta and evaluate it by histological analysis, scanning electron microscopy, and X-ray diffraction. The placenta was decellularized chemically and then lyophilized by a lyophilizer (FTS Systems Bulk Freeze Dryer Model 8–54) for 24 hours at -56 °C and 5 mm Hg until they were totally dried. The assessment used histological analysis, Scanning Electron Microscopy, and x-ray diffraction. The hematoxylin and eosin stain demonstrated that cellular populations and nuclear residues were totally absent from HP tissue. The freeze-drying process of preparing acellular human placenta powder resulted in structures that are made up of highly interconnected, open networks of pores. The particle size mean diameter was approximately ranging from a minimum of 89.44 µm to a maximum of 172.82 µm, and the pore sizes ranged between 44.28 µm and 81.40 µm. Using conventional diffraction database cards, the X-ray diffraction analysis of acellular human placenta powder demonstrated the existence of the constituent organic and inorganic components. It was discovered that the presence of semi-crystalline or amorphous organic components, such as chondroitin sulfate, collagen, and hyaluronic acid. The study concluded from the structural powder that it can be used in regeneration treatments such as treating the spinal cord in animals.

Keywords

powder, X-Ray Diffraction, Extracellular matrix, Scanning Electron Microscopy, histological analysis

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Abbreviations

HP: Human placenta
ECM: Extracellular matrix
SEM: Scanning Electron Microscopy
XRD: X-Ray Diffraction

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The human placenta is an easily accessible, affordable, and morally acceptable source of raw material because it is a transient important organ that is often disposed of as medical waste. Apart from its accessibility, the placenta has other advantageous biological characteristics that are intrinsic to the healing process, such as angiogenic capabilities [1,27], anti-inflammatory properties [2], antibiotic use [3], antifibrotic [4,29], and immunomodulatory [5] with low immunogenicity [6,29]. Placental tissues feature a special extracellular matrix (ECM) with remarkable mechanical and structural characteristics, including tensile strength, stiffness, and elasticity, which complement the desired biological traits [7,17]. As mentioned in the preceding section, the extracellular matrix's (ECM) composition differs depending on the source. Placental-derived biomaterials with living cells, in addition to the ability to function through paracrine pathways, have the above-described qualities[8,33].

The cells found in placental-derived biomaterials facilitate the release of trophic factors, which aids in tissue repair [9,34] and immunomodulation [10]. Furthermore, placental-derived biomaterials' growth factors and cytokines promote anti-inflammatory and antibacterial activities [11,35]. There are several ways to make placental-derived biomaterials, such as using the umbilical cord. (including umbilical cord blood, umbilical cord tissue, and Wharton's jelly), the amniotic sac (including amnion and chorion), amniotic fluid, or a combination of these sources [12]. This produces a great deal of biomaterials but also adds a great deal of variety. The compositions of different sources vary. For example, the extracellular matrix (ECM) of the amniotic membrane is rich in collagen and includes a range of bioactive ECM components, including glycosaminoglycans, fibronectin, laminin, and elastin [13]. While Wharton's Jelly is a mucoid connective tissue composed of a network of glycoprotein microfibrils and collagen fibrils [14,30]. Furthermore, studies have demonstrated that the immunomodulatory qualities vary on the source [15,31]. Even when comparing biomaterials from the same source, there is variation both between and within the donors. [16,32]. The objective of the present study was to prepare a powder from the human placenta and evaluate it using X-ray diffraction and scanning electron microscopy. X-ray diffraction (XRD) was used to study the powdered acellular human placenta in more detail. The Joint Committee on Powder Diffraction Standards (JCPDS) maintained the Powder Diffraction File (PDF-2) from the International Center for Diffraction Data, where standard diffraction data were compared to the XRD patterns.

The acellular human placenta powder contained a variety of organic and inorganic elements, as shown by the analysis of the detected peaks. The following elements were specifically identified as the causes of the peaks:

1. PDF#83-1494 (Chondroitin Sulfate)
2. PDF#50-2241 (Collagen)
3. JCPDS#09-0432 (Hyaluronic Acid)

Abrupt and powerful peaks were seen at 2θ = 7.97°, 11.57°, and 19.13° when certain semi-crystalline or amorphous organic components, such as hyaluronic acid (HA), collagen (Col), and chondroitin sulfate (CS), were present.

The results of the XRD analysis confirmed that the main extracellular matrix (ECM) components, including collagen, hyaluronic acid, and chondroitin sulfate, were present in the decellularized human placenta powder. Understanding the structural and compositional properties of the acellular placental scaffolds is important since they may be used in a wide range of tissue engineering and regenerative medicine applications.

Histological examination was performed in order to evaluate the impact of employing chemical agents in decellularization human placenta (HP). Specifically, hematoxylin and eosin staining was used for confirmation of the decellularization process is illustrated in (Fig. 1 A & B). The staining demonstrated that cellular populations and nuclear residues was totally absent from HP tissue.

One important parameter that can have a big influence on how well biological implants work is the pore width of tissue engineering scaffolds. The porous design of these scaffolds is often the result of water evaporating during the lyophilization (freeze-drying) process, which can lead to the fragmentation of the scaffold's interior particles.

Examination of prepared scaffolds was done by Scanning Electron Microscopy (SEM) revealed differences in particle size and pore size in the bioscaffolds, acellular human placenta powder that was prepared by freeze dry method gave rise to structures that are consisted of open networks of pores with a high degree of interconnection, the particle size mean diameter was approximately ranging from minimum of 89.44 µm to maximum of 172.82 µm (Fig. 2 A), and pore sizes that ranged between 44.28 µm and 81.40 µm (Fig. 2 B).

The mean pore size has a crucial role in the dis-

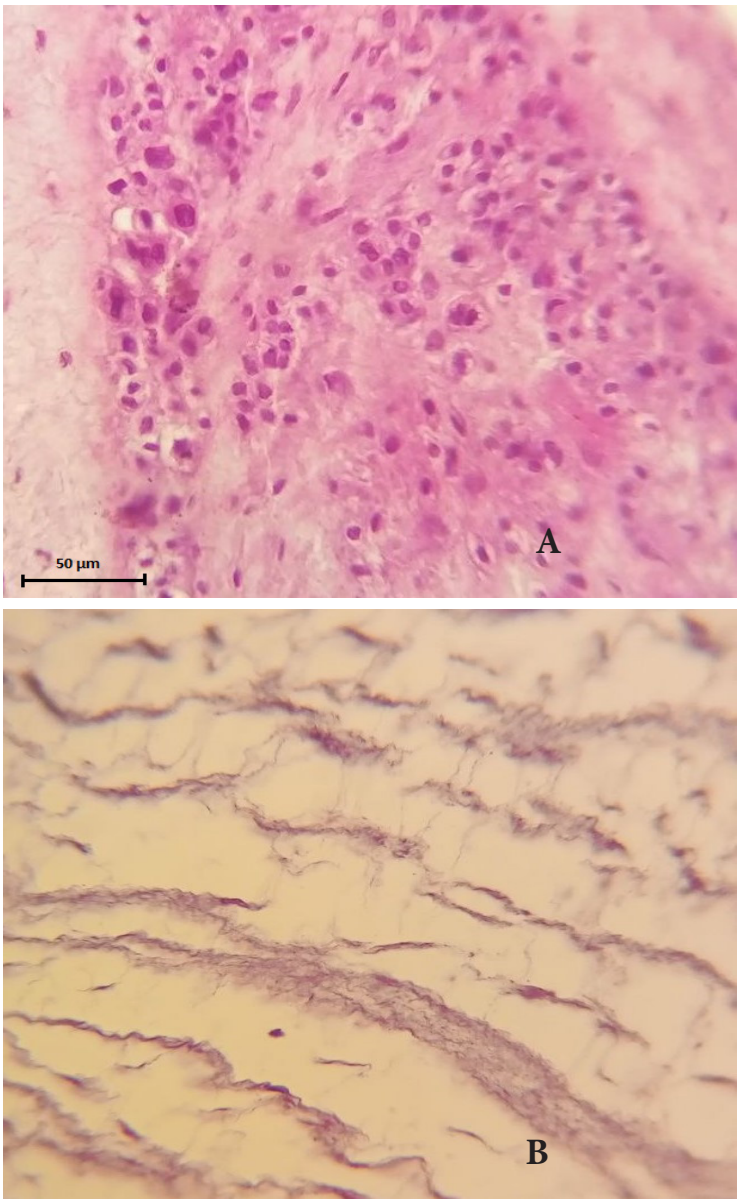

Figure 1.
(A) In the native human placenta shows cell nuclei often arranged in isogenic groups, and evenly distributed in the ECM. (black arrows) (B) Decellularized HP complete loss of cellularity (H&E X200).

tribution of nutrients and removal of waste materials from scaffolds used in tissue engineering. The ability of cells to migrate toward the center of the construct may be restricted by too-small holes, which may obstruct these essential processes. However, the precise relationship between scaffold pore size and cell activity remains unclear. [20]

Similar results on the porosity structure of scaffolds made by freeze-drying have been reported in several prior studies. [21] The morphological structure of urinary bladder powder obtained by freeze-drying is characterized by large interconnected pores in the range of 20 to 100 µm. This pore size range seems to be suitable for certain applications, such as long-size peripheral axon regeneration, which requires pores ranging from 200 to 750 µm, or even up to the millimeter scale. In contrast, smaller pores (20-70 µm) may be more appropriate for the ingrowth and extension of long peripheral axons.

Interestingly, scaffolds with pore diameters around 100 µm have been proposed to be more suitable for neuronal regeneration, depending on the source of the biomaterial [22]. However, according to a study by Hausner et al. 2006, Schwann cells transplanted on fibrin-coated polyurethane scaffolds with a uniaxially-oriented pore structure that is, with 2 µm hole walls and 75 × 750 µm elongated pores showed a significant amount of peripheral axon regeneration [23].

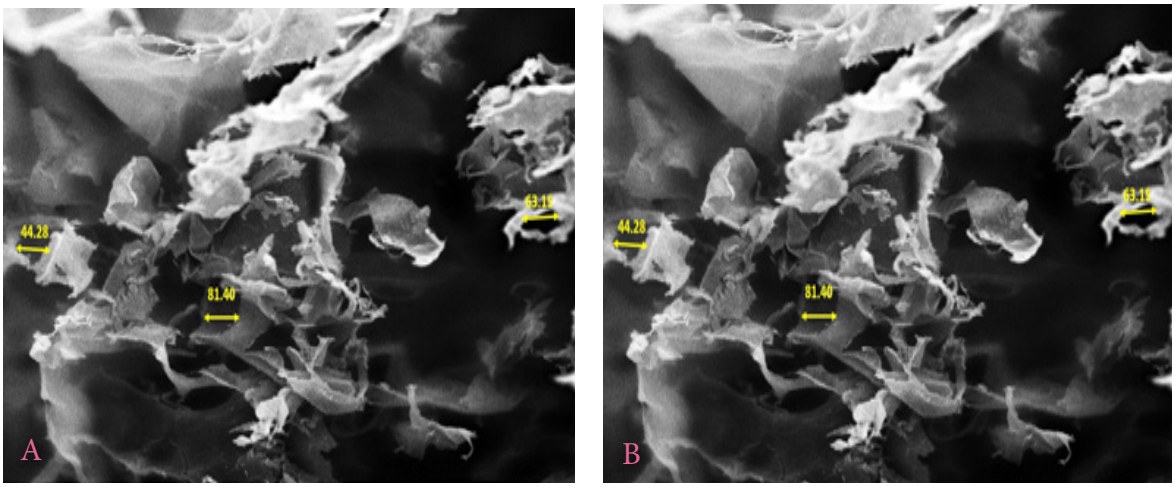

Figure 2.
SEM micrograph the structural morphology of the prepared bioscaffolds shows (A&B) acellular human placenta powder produced by lyophilization.

Additionally, parallel studies [24, 28] have shown that increasing the groove width from 50 to 200 µm in poly(dimethyl siloxane) scaffolds coated with essential proteins like poly-L-lysine and laminin can improve axon regeneration.

In summary, the pore size and architecture of tissue engineering scaffolds play a critical role in facilitating cell infiltration, nutrient/waste exchange, and ultimately, the desired tissue regeneration outcomes. The optimal pore size can vary depending on the specific application and the source of the biomaterial, highlighting the importance of carefully designing and characterizing scaffold properties to achieve the desired biological performance.

The standard diffraction database cards (PDF-2) from the International Center for Diffraction Data were compared to the X-ray diffraction (XRD) examination of the acellular human placenta powder. Diffraction peak analysis revealed that the placenta scaffold includes several significant organic and inorganic components. Specifically, the abrupt and exceptionally potent peaks at 2θ = 7.97°, 11.57°, and 19.13° were accounted for by the presence of semi-crystalline or amorphous organic materials, namely chondroitin sulfate (CS), hyaluronic acid (HA), and collagen (Col). Several previous studies have strongly supported these results. [25].

Scanning techniques such as X-ray diffraction (XRD) provide valuable insights into the crystalline phase and unit cell dimensions of the scaffold materials, making them ideal for the characterization of tissue engineering scaffolds. In the case of the acellular human placenta powder, the XRD analysis confirmed the presence of significant extracellular matrix (ECM) components, including collagen, laminin, fibronectin, proteoglycans, and elastin.

The process of removing cells from scaffold tissue while preserving the extracellular matrix (ECM) structure is known as decellularization. It may be transported physically or chemically. This extracellular matrix (ECM) provides a three-dimensional framework for nerve regeneration and other tissue engineering applications [26].

ECM components such as collagen, chondroitin sulfate, and hyaluronic acid need to be identified by XRD analysis. Within the scaffold, these chemicals are crucial for maintaining cell adhesion, encouraging structural support, and enabling cellular communication and differentiation. To keep the scaffold bioactive and suitable for use in tissue engineering applications, some crucial extracellular matrix elements must be preserved throughout the decellularization process.

In summary, the XRD analysis of the powdered, acellular human placenta provided crucial information on the presence and composition of the scaffold's primary organic and inorganic constituents. Understanding a scaffold's structural and functional properties is essential for determining its potential applications in tissue engineering and regenerative medicine. This understanding is derived from the scaffold's composition.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

Authors' Contributions

HA conceived and planned the experiments. JMK contributed to sample preparation. In addition to leading the paper writing effort and offering insightful criticism, HA and JMK helped analyze the data and shaped the study, analysis, and manuscript.

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