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A Review of Hemocompatibility of Bio Scaffolds for Bone Regeneration: An In Vitro Assessment Using Human Red Blood Cells

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ABSTRACT:
To facilitate healing and reconstruction of bones, the bone regeneration frame is used more and more in orthopaedic and jaw procedures. Hamo-bonds of the ability of the frame that are favourable with the blood are important, but often ignore the components of biocompatibility. Haematology, thrombosis, inflammation and ultimately implant deficiency can be caused by interactions with blood components, especially red blood cells. Focusing on testing in tests using human red blood cells, this article explains recent studies on bioscaffold for bone regeneration. We perform a variety of materials for frames, how to assess RBC compatibility and possible approach to enhance hem -binding possibilities. The design and description of biocompatible frames that successfully integrate and successfully and support bone regeneration requires a deeper understanding of these interactions.

Keywords:
Bone regeneration, Bio scaffold, Biocompatibility, Red blood cells, Hemocompatibility.

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INTRODUCTON

The main clinical challenge is bone defects due to disease, trauma, or surgical resection. The regeneration of bone based on biomaterials has recently interested in the potential substitute for autografts and allografts. These scaffolds provide a three-dimensional (3D) matrix, which contributes to the cell adhesion, growth, and differentiation while directing the development of new bone. Biocompatibility, Biodegradability, osteo conductivity, osteoinductivity, and suitable mechanical strength are all preferable in a scaffold (Hutmacher et al., 2000).

Hemocompatibility, or a material's ability is to interact favourably with blood, and it is generally ignored when designing and evaluating scaffold, while biocompatibility frequently includes cytotoxicity, inflammation, and tissue integration. The bio scaffold instantly comes into contact with blood after implantation, starting a complicated chain of events. The coagulation, complement activation, platelet adhesion and aggregation, and most importantly haemolysis and damage to red blood cells (RBCs) can all result from this interaction (Anderson et al., 1999). During haemorrhage, the RBC is damages and releasing haemoglobin and intracellular materials into the surrounding environment. These liberated substances can prevent bone

regeneration and cause oxidative stress and inflammation (Tuma et al., 2014).

Thus, it is important to evaluate the bone regeneration scaffolds, especially the method of interacting with RBCs. As an indicator of the blood and material interactions and, this paper reviews the bone regeneration, and use of human red blood cells.

Bone tissue engineering is a multidisciplinary field focused on repairing or replacing damaged bone tissue through a combination of biomaterials, cells, and growth factors (Bose et al., 2012; Mohamed & Shamaz, 2015). Scaffolds play a crucial role in this process by providing a three-dimensional structure that supports cell adhesion, proliferation, and differentiation, guiding new bone tissue formation (Chocholata et al., 2019).

In bone regeneration, scaffolds serve as a template for bone ingrowth, providing mechanical support and delivering bioactive cues to stimulate bone formation (Chocholata et al., 2019). The ideal scaffold should be biocompatible, biodegradable, and possess a suitable pore size and architecture to facilitate vascularization and nutrient transport (Dalfino et al., 2023; Sabir et al., 2009).

Red blood cells, primarily known for their oxygen-carrying capacity, have emerged as a promising component in bone tissue engineering due to their potential to enhance vascularization and promote bone regeneration (Guo et al., 2023). Vascularization, the formation of new blood vessels, is essential for the survival and function of newly formed bone tissue, ensuring an adequate supply of oxygen and nutrients while removing waste products (Balmayor & Griensven, 2015).

The incorporation of red blood cells into scaffolds can promote angiogenesis, accelerating the bone regeneration process. Stem cells are often combined with biomaterials, scaffols, and growth factors to promote bone healing at fracture sites, showcasing the importance of cellular components in bone regeneration strategies (Iaquinta et al., 2019). Mesenchymal stem cells have demonstrated effectiveness in renewing bone tissue defects, highlighting their crucial role in regenerative processes (Diomede et al., 2020).

However, the integration of red blood cells into scaffolds presents significant hemocompatibility challenges, as the introduction of blood components can trigger adverse reactions such as thrombosis, inflammation, and immune responses (Guo et al., 2022). Hemocompatibility refers to the ability of a material to interact with blood without causing harmful effects (Perez et al., 2018).

SCAFFOLD MATERIALS AND THEIR HEMOCOMPATIBILITY

Various materials are employed in the fabrication of bone regeneration scaffolds, each exhibiting different levels of hemocompatibility.

Collagen: A protein that occurs naturally and makes up a significant portion of the extracellular matrix (ECM), collagen is frequently utilised in bone regeneration. Usually, type I collagen is used. In general, collagen scaffolds are biocompatible and biodegradable. However, a few research has established that collagen can cause platelet aggregation and promote the coagulation cascade (Woodhouse et al., 2003). Its hemocompatibility may be affected by purification ranges and crosslinking strategies.

Biodegradable synthetic polymers with a range of mechanical characteristics and rates of degradation include poly (lactic acid) (PLA), poly (glycolic acid) (PGA), poly (lactic-co-glycolic acid) (PLGA), and polycaprolactone (PCL) [5]. The type of polymer, molecular weight, and surface properties all affect their hemocompatibility. In general, hydrophilic polymers are greater hem compatible than hydrophobic ones. Their blood compatibility may be enhanced by surface change the usage of hydrophilic polymers which includes polyethylene glycol (PEG) (Lee et al., 1995).

IN VITRO ASSESSMENT OF RBC COMPATIBILITY

A number of invitro techniques are employed to assess the hemocompatibility of bioscaffold using human red blood cells. These tests offer

important information about the possibility of RBC damage and its after effects.

The most popular technique for determining RBC compatibility is the haemolysis assay. A suspension of human red blood cells in a physiological solution is incubated with the bioscaffold material for a predetermined amount of time, usually one to three hours. The amount of released haemoglobin is determined using spectrophotometry after the supernatant is collected following incubation. The amount of haemoglobin released in comparison to a positive control (complete lysis) and a negative control (no lysis) is used to compute the percentage of haemolysis. Guidelines for performing haemolysis assays are provided by the ASTM F756 standard. Important parameters that require careful control include the ratio of material surface area to blood volume, incubation duration, and RBC concentration.

FACTORS INFLUENCING RBC COMPATIBILITY

The relationship between RBCs and bio scaffolds is influenced by a number of factors:

Material Composition:
The chemical composition of the scaffold material is a significant component. As was previously indicated, RBC lysis can be caused by the surface charge or degradation products of some materials, such as synthetic polymers and some bioactive glasses.

Surface Properties:
RBC adhesion and activation are greatly influenced by surface features such as surface roughness, hydrophobicity/hydrophilicity, and surface charge. RBC adhesion is generally higher on rougher surfaces (Bagherifard et al., 2015). When compared to hydrophilic surfaces, hydrophobic surfaces frequently show reduced hemocompatibility.

Porosity and Pore Size:
Blood flow and RBC interaction may be impacted by the scaffold's porosity and pore size. RBC mobility may be restricted by smaller pore sizes, which may result in cell damage.

Sterilisation Techniques:
Sterilisation techniques like autoclaving, gamma irradiation, and ethylene oxide sterilisation can change the scaffold material's surface characteristics and impact its hemocompatibility (Kulkarni et al., 1966).

STRATEGIES TO IMPROVE HEMOCOMPATIBILITY

Several strategies can be employed to improve the hemocompatibility of bone regeneration scaffolds:

Surface Modification: Protein adsorption and RBC adhesion can be decreased by covering the scaffold surface with biocompatible substances like PEG, hyaluronic acid, or chondroitin sulphate (Unsworth et al., 2005)
Material Selection: RBC interaction can be reduced by selecting materials with built-in hemocompatibility, like collage or specific bioactive polymers.

Incorporation of Antithrombotic Agents: heparin or nitric oxide donors are examples of antithrombotic agents that can be incorporated into the scaffold can inhibit coagulation and reduce the risk of thrombosis (Kim et al.,2011).

Texturing and Surface Engineering: RBC adhesion and activation can be influenced by altering the surface texture and creating micro- or nano-patterns.

Optimization of Porosity and Pore Size: By carefully regulating the scaffold' porosity and pore size, blood flow can be enhanced and RBC damage can be reduced.

CONCLUSION

A key consideration in the design of bio scaffolds for bone regeneration is hemocompatibility. Predicting the scaffold's reaction in the intricate biological environment requires evaluating the interaction between scaffolds and human red blood cells using in vitro assays. Together with RBC morphology analysis, aggregation studies, and ROS measurement, the haemolysis assay offers important insights into the risk of RBC

damage and its ensuing negative consequences. In order to create biocompatible and efficient scaffolds for bone regeneration that support successful bone healing and reconstruction, it is essential to comprehend the variables that affect RBC compatibility and use techniques to increase hemocompatibility. In order to validate the results of in vitro studies, future research should concentrate on creating increasingly complex in vitro models that replicate the intricate in vivo environment and integrating in vivo hemocompatibility assessments.

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