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Hybrid polymer film preparation as a scaffold for tissue engineering and its mechanical strength

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Abstract
Biodegradable polymers are the most preferred material as a scaffold for tissue engineering. Synthetic biodegradable polymers exert good mechanical properties. Therefore, combining them with natural polymer has an advantage, as they would build good platforms with adequate biocompatibility and suitable mechanical property for tissue engineering. However, processing temperature influences the properties of the composite and the ability to add bioactive molecules such as drugs or growth factors. Therefore, an attempt was made to engineer a synthetic and hybrid scaffold using the solvent casting method, wherein the scaffold would be prepared at relatively lower temperatures.

Keywords: Bio-scaffold, polyurethane, poly l lactic acid, solvent casting polymer, polymer mechanical property

Introduction
In terms of absorbability and stability, biopolymers can be divided into two groups - Biodegradable polymers and Non-biodegradable polymers. Non-biodegradable polymers are not usually applied in tissue engineering as they do not meet an ideal scaffold's criteria and do not degrade in the physiological environment.

Biodegradable polymers, which are the most preferred material as a scaffold for tissue engineering, can be further classified in terms of their origin as natural and synthetic. [Yuan *et al.* 2008] [1].

The tissue engineering paradigm mainly involves cells, scaffolds, and growth factors or cues for maintaining cell function and growth. The scaffold is the framework provided for the tissue formed to attain its 3-dimensional shape ideally mimicking the native extracellular matrix. When designing an artificial matrix, one must consider mechanical strength and the construct's ability to provide a local environment that encourages tissue growth and discourages infection and excessive immune responses. Matrices must be more than simply biocompatible to be an effective vehicle for tissue engineering applications [Nge *et al.* 2010] [2]. Hence an effort was made to engineer a synthetic scaffold using the solvent casting method. Solvent casting is a method to fabricate a macroscopic formulation that can be implanted or inserted for long-term medication [Rabin *et al.* 2008] [3].

Keratin is well known as a biocompatible and biodegradable protein [Yamauchi *et al.* 1996] [4], which can accelerate the growth of fibroblast [Yamauchi *et al.* 1998] [5]. Therefore, keratin should be applied to biomedicine, just like collagen and fibroin. Unfortunately, the poor mechanical properties of regenerated keratin hinder its processability and limit its practical applications in blending with suitable polymers with better structural properties. [Xing *et al.* 2011] [6].

Amino acids in the keratin expose various functional groups, *viz*., Amide (NH2), Carboxyl (COOH), Hydroxyl (OH), and Sulfhydryl (SH) which acts as a bio-reactive site for the attachment of molecules of interest- drugs, growth factors and other such compounds [Khosa and Ullah, 2013] [7].

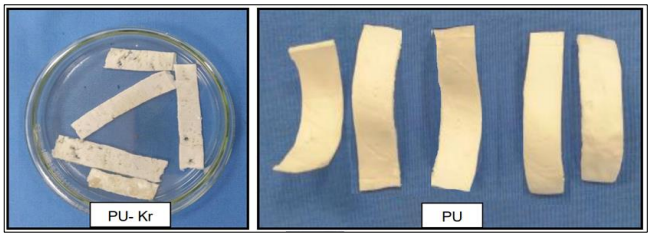
The following experiment was made to create a solvent cast composite film that can be used in tissue engineering and compare its mechanical strength. Five specimens of each composite and synthetic polymer were prepared by the following method and subjected to mechanical testing in the universal testing machine.

Materials and Methods

Two polymers were chosen to design the film, Polyurethane [Selectophore ™] from Sigma Aldrich and Poly L Lactic Acid (PLLA) From Nomisma Health care. Solvents used were Dimethyl sulfoxide (DMSO) And Chloroform sourced from Qualigens Fine Chemicals Pvt. Ltd. Keratin was obtained from chicken feathers and processed according to the method described by Ayutthaya *et al.* 2015[8].

1. Preparation of polyurethane films

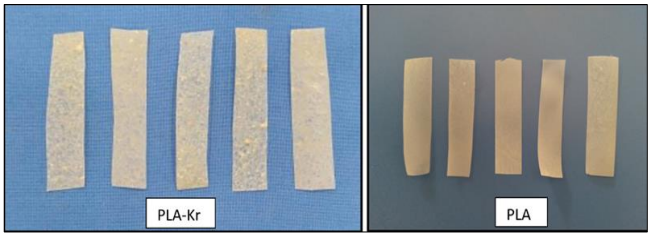
Two grams of Polyurethane dissolved in 5ml DMSO at the temperature of 50 ℃ for three days, and Polyurethane with keratin (50%) was blended with a homogenizer. Then it was cast between a glass plate with spacers of 1 mm thickness. Then the sheets were washed thoroughly in cold distilled water multiple times.



Polymer Composite PU-Kr and PU

2. Preparation of PLLA films

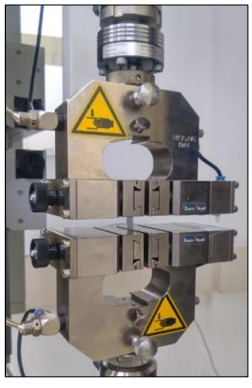
Four grams of PLLA and PLLA with keratin (50%) were dissolved in 20 ml Chloroform for 30 min at room temperature and cast on the Petri plate to obtain a thin film after evaporation of chloroform.



Polymer Composite PLA-Kr and PLA

3. Mechanical testing

Five specimens prepared from each method were taken up for testing according to ASTM D882 standards in Zwick/Roell Z030 UTM in TÜV Rheinland (India) Pvt. Ltd., Bengaluru, under the test conditions of gauge length of 15 mm and a test speed of 1 mm/sec.



Mechanical testing machine setup

Results and Discussions

1. Polyurethane films

Polyurethane (PU) emerged as another synthetic polymer that meets the medical device market's demands for thinner wall sections, longer lengths, and extended blood exposure. Both biostable and biodegradable polyurethanes with various mechanical properties and porosities can be obtained by careful selection of raw materials and processing conditions.

PU is commonly used in long-term implants such as cardiac pacemakers, wound care vascular grafts, orthopedics, urology, and tissue replacements. Though Extrusion or injection molding is the most common practice for designing these, the solvent casting method can also be employed for this purpose.

Solvents used to dissolve PU must be highly polar organic solvents such as N-Dimethyl formamide (DMF)/ Tetrahydrofuran (THF), 1, 1, 1, 3, 3, 3,-hexafluoro-2-propanol (HFIP) or combination solvent system. These are highly toxic, and the smallest of the traces left in the scaffold can lead to an unfavorable consequence. DMSO is a reasonably safe alternate.

Ikeda *et al.* (2014) [9] suggest that the scaffold made from biopolymer for tissue engineering should have a mechanical strength between 0.03 and 0.06 MPa. Preferably, the scaffold should have minimum mechanical strength, be reliable with the anatomic site, into which it gets to be implanted, and must be strong enough to allow *in vitro* or *in vivo* handling during implantation (O'Brien, 2011) [10]

Mechanical properties of biomaterials and tissue extracellular matrix (ECM) are among the most imperative factors in tissue engineering. Namely, the elastic modulus, or stiffness of biomaterials and tissue ECM vary in a broad range between 3 kPa and several GPa (Seliktar 2012 and Shakoor *et al*., 2013) [11, 12]

The elasticity of the solvent cast polyurethane was halved after adding the keratin, while the ability to handle stress was reduced by four times (Table 2). The mechanical strength of the composite was subpar by the slightest margin when that of PU alone was ideal for porous scaffold, as described by O'Brien, 2011 [10].

2. Poly L Lactic Acid (PLLA) films

In 1970, PLA products have been approved by the US Food and Drug Administration (FDA) for direct contact with biological fluids. PLA has an extensive mechanical property profile and is thermoplastic with high biocompatibility and biodegradability (Gupta *et al*. 2007) [13].

PLA has a broad spectrum of applications, there are certain limitations such as i) slow degradation rate, which could be up to years, ii) hydrophobicity leading to low cell affinity, and can elicit, in some cases, an inflammatory response from the living host upon direct contact with biological fluids. iii) brittleness, with less than 10% elongation at break, and iv) Lack of reactive side-chain groups - chemically inert with no reactive side-chain groups makes its surface and bulk modifications challenging. PLA is thermally unstable and exhibits rapid loss of Mw and consequent erosion of its mechanical properties as well. (Farah *et al*. 2016) [14]

Blending PLA with other polymers offers convenient options to improve associated properties or to generate novel PLA polymers/blends for target applications.

Good solvents for PLA products are dioxane, acetonitrile, chloroform, methylene chloride, 1, 1, 2-trichloroethane, and dichloroacetic acid (Ebrahimi and Ramezani, 2021) [15].

The elongation percentage was less than 10% than that of the

PLA film produced by any other method (Farah *et al*. 2016) [14]. Adding keratin to PLA didn't change the stress or strain calculations. Though the material was very brittle, it was tough and could handle substantially higher force with and without the addition of keratin (Table 2).

Statistical Analysis

Synthetic polymer alone when used to prepare film using solvent casting method showed excellent load bearing capability. This load bearing is marginally decreased when blended with kearatin in case of PLA, substantially with PU.

Table 1: Mean, Sample Standard Deviation (SD) and Standard error of mean of specimens

Specimen	Max. Load, N			Elongation @break, %		
	Mean	Sample Standard Deviation (SD)	Standard error of mean	Mean	Sample Standard Deviation (SD)	Standard error of mean
PU	43.0	14.8	7.4	751	203.11	101.55
PU+Kr	11.9	5.15	2.30	360	125.11	55.95
PLA	30.8	16.26	7.27	7.1	1.48	0.66
PLA+Kr	27.5	4.32	1.93	6.6	0.55	0.24

Addition of the Keratin to the synthetic polymer increased the uniformity of the specimen prepared which is evident with lower Sample Standard Deviation (SD) and standard error of mean (Table 1) in those specimesaa.

Table 2: Stress and strain calculations of polymer and polymer composites

		PU	PU+Kr	PLA	PLA+Kr
Stress calculations					
Area (A)	cm^2	1.5	1.5	1.5	1.5
Force (F)	N	43	11.9	30.8	27.5
Stress (σ)	Mpa	0.286667	0.079333	0.205333	0.183333
Strain Calculations					
Initial Length (L1)	cm	1.5	1.5	1.5	1.5
Final Length (L2)	cm	12.765	6.9	1.6066	1.6
Percent elongation	(%EL)	751	360	7.1	6.6
Change in length (ΔL)	cm	11.265	5.4	0.1066	0.1
Strain (ε)		7.51	3.6	0.071067	0.066667
Youngs Modulus (E)	Mpa	0.03817	0.022037	2.889306	2.75

Conclusions

This study aims to create a framework from synthetic and natural polymer, remove its limitations, and enable the production of highly characterized materials with the mechanical properties required for tissue engineering applications. For the tissue engineering application where elasticity is required PU blended with keratin may be utilized and in an application where rigidity is required, with strong load bearing capacity, PLA blended with keratin can be used.

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