Investigation of Biological Activity, Open Porosity and Water Absorption of Ternary Polymeric Blend (Polymethylmethacrylate / Starch) used for Bone Scaffold

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Summary

In this study a fabrication of three groups of biological system for bone scaffold, which consist of first group samples (Acrylic bone cement: % X poly methyl meth acrylate), second group samples (poly methyl meth acrylate: % X Acrylic bone cement), with selected weight percentages of second material are (5, 10,15 and 20 % wt.) and third group, (Acrylic bone cement: 15 % poly methyl meth acrylate : % X starch) with selected weight percentage ratios of starch are (2, 4 and 6 %wt.). In vitro biological activity and water absorption have been studied. The results clarified that the in vitro biological activity test showed that all fabricated samples have no biological influence on the gram positive bacteria Bacillus subtilis and gram negative bacteria *Escherichia coli* . The results show that the first group of polymer blend have low open porosity and water uptake capacity than a second group. The increasing in starch weight fraction for the third group led to increasing in open porosity and water absorption..

Keywords: Broiler poly methyl meth acrylate, Starch, Water absorption, Biological activity.

Introduction

Bones play an important role in our bodies, providing the ability to perform daily activities. They are also responsible for mineral storage and red blood cell production. When bones fail to repair themselves due to severe injury or disease, a replacement surgery can be performed to replace the bone defect with a bone substitute (i.e., Bone, as a functionally smart tissue, is capable of healing and remodeling in the case of limited bone defects) (1 and 2) .

Poly methyl meth acrylate (PMMA) is non-adhesive acrylic polymer that is widely used in orthopedics as bone cement for implants. The orthopedic use of PMMA cement was introduced in the early 1960s by Chanley and this was the first cement used for spine applications. PMMA has been widely investigated as a bone cement for over 40 years and many injectable PMMA cement formulations designed for vertebral body applications are commercial available. PMMA bone cement occupied an unquiet space for several decades due to its desirable characteristics, e.g. sufficient strength to provide mechanical support, moldable to fill complex defects, (3 and4). A polymer blend is a member of a class of materials in which two or more polymers are blended together to create a new material with different physical properties. They combine in an advantageous manner the properties of the alloying components and in some cases the properties of the blends polymeric are superior to those of their individual components, blending of polymer is a technological way for providing materials with full set of desired specific properties at the lowest cost, e.g. a combination of strength and toughness, strength and solvent resistance, etc. Blending also benefits the manufacturer by offering improved process ability, product uniformity, quick formulation changes, plant flexibility and high productivity, i.e. Polymer blending is one of the most common techniques employed for developing new polymeric materials [5]. Starch is one of the most abundant and cheap polysaccharides produced by various plants as a source of stored energy. It is found in plant roots, stalks, and. crop seeds and is produced by staple crops such as rice, corn, wheat, tapioca, and potato. It is deposited in the chloroplast of plant cells as insoluble granules composed of α-amylose (normally 10–30%) and amylopectin(normally 70–90%), depending on its source. Starch granules are hydrophilic and show strong intermolecular associations through hydrogen bonds formed by hydroxyl groups on the granule surface. Starch-based materials have been widely used as biomaterials in bone replacement and fixation and for developing tissue engineering scaffolds. In addition, some studies have shown that starch is beneficial in drug delivery applications [6]. In most studies, PMMA was used as a matrix material alone and was reinforced by some substances such as (fly ash, fly dust and ZrO2, and Y2O3) or a binary phase composition of PMMA and other polymeric materials such as HDPE and the mechanical and biological characteristics was studied for them (7, 8 and 9). The aim of this study is to prepare three type of polymeric blends with PMMA as the base material which was used to improve antibacterial activity of biomaterials, firstly via blending different types of PMMA material, secondly via blending another type of chemically different polymers with optimum sample of the first groups as a tool for tailoring characteristics of the products with optimized material properties.

Materials and methods

Preparation of Blends: Acrylic bone cement for bone scaffold, as resin material manufactured by (company of Re- Acromed) was supplied from UAE, and another type of acrylic resin which is PMMA manufactured by (ORT-365) from Ankara. Table (1and 2) show the technical information of acrylic bone cement (Type: Re-Acromed) and PMMA (Type: ORT-365) respectively according to the product company

Table, 1: Technical information of acrylic bone cement according to the product company.

Moulding Consistency	
Mixing Ratio	5 wt. powders, 3.5 wt. liquid
Mixing Time	Approximately 30 seconds
Working Tim	Ends after about 2 minutes
Curing Time	Approximately 14 minutes

Table, 2: Technical information of PMMA according to the product company.

Viscosity at 20℃	300/350 mPa.s
Resin/ Hardener	100/2
Time for Work at 20℃	8-10 min.
Time for separation	15 min
Hardness shore D	80-85

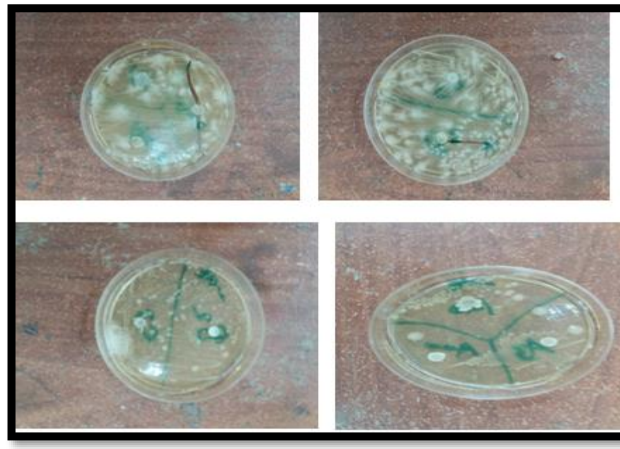
Corn starch, the starch that was utilized in the current work was obtained from Daejung chemicals and metals Co., LTD (Korea). Table,illustrated the properties of starch that's used in present research according to the product company

Table, 3: Properties of starch according to the product company.

Formula	$C_6H_{10}O_5$
pH	4-7
Moisture Content	≤ 15%

Acrylic bone cement specimens were prepared by hand lay-up molding (5 wt. powders) with (3.5 wt. liquid), of methyl methacrylate monomer, as well as according to manufacturer instructions for PMMA, the mixing ratio of acrylic resin to hardener is (100/2). Two types of polymeric blends were prepared. The first type were prepared by weighing amount of acrylic bone cement as a basic substances reinforced by (5, 10, 15 and 20 %wt.) of PMMA were uniformly mixed manually until the mixture got to homogenous state. The mixture was poured into the mold properly to get the final uniform specimens. After the molding process, for the purpose of facilitating the exit of samples from it, also to get smooth sample surface and free from defect. This mixture has been left in the mold for 48 hours at room temperature to solidify it. After that, the cast is placed inside an oven at 50 ºC for 3 hours. Finally, it was left for 72 hours at room temperature. This process is important to reveal complete polymerization. To prepare the second type of polymer blends, the same pre-preparation steps were repeated except for the proportions of the mixing components mentioned above, they have been replaced by one another. A ternary phases of polymeric materials were prepared by blending (Acrylic bone cement: 15 % PMMA) with (2, 4, and 6) % wt. of starch.

In vitro Biological Activity Test: The antibacterial activity of acrylic bone cement, PMMA, (Acrylic bone cement: 15% PMMA), and (Acrylic bone cement: 15 % PMMA: 2% starch) Specimens, were carried out by disk diffusion method, with a (6 mm) diameter and (2 mm thickness. The bacterial microorganism which were utilized in this test include gram positive bacteria Bacillus subtilis and gram negative bacteria Escherichia coli (E. coli). Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultured were swabbed on these plates. All samples were placed in the nutrient agar plate and kept for incubation at 37 ºC for 24 hours. Zone of inhibition were measured. The experiments were repeated four times and mean values of zone diameter were presented,(Fig.1) illustrates zone of bacterial inhibition by prepared disk



Figure, 1: Shows zone of bacterial inhibition.

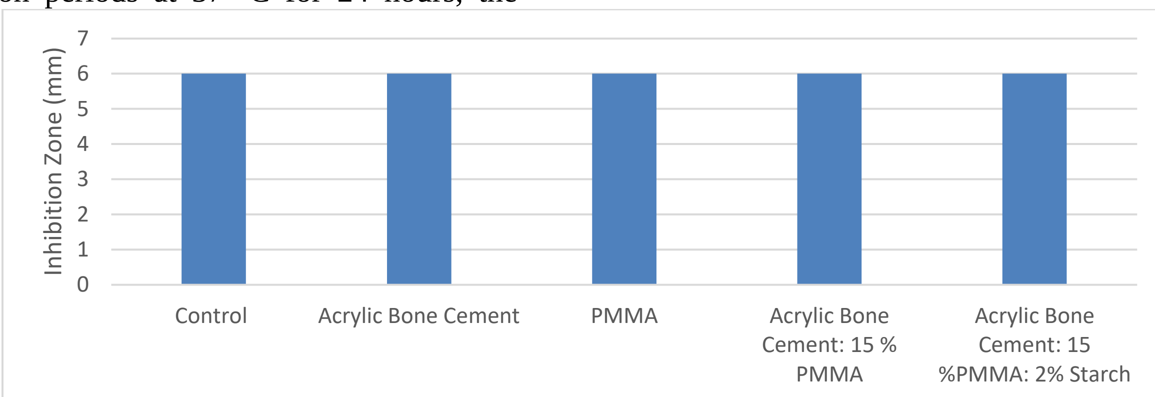
Water Absorption and Open Porosity Test: According to ASTM D-570 [10], the water absorption and open porosity was carried out by utilizing (Precision balance), manufactured by (Radwag in USA), kind (series) (PS 360/C/1). In this test the rectangular samples with dimensions (10 mm) width, (20 mm) length, and (4 mm) thickness, were immersed in deionized water for 30 days [11]. Water absorption and open porosity test was performed for all fabricated samples by weighing the specimens in three different conditions using a four digit weight scale: The first weight in air. The second weight includes immersion of the specimens through a hook balance in distilled water. In the third weighing, the specimens were immersed in distal water for 30 days, and during this period the specimens were removed from water at each 24 hours and dryness by using soft cloth, then weighed by using electronic balance of accuracy 10-4. The percentage of water uptake capacity and open porosity percentage were calculated from the following equations (12).

$$\%\text{Water Absorption} = \left(\frac{\text{Wet weight} - \text{Dried weight}}{\text{Dreied weight}}\right)*100 \quad .... [1]$$

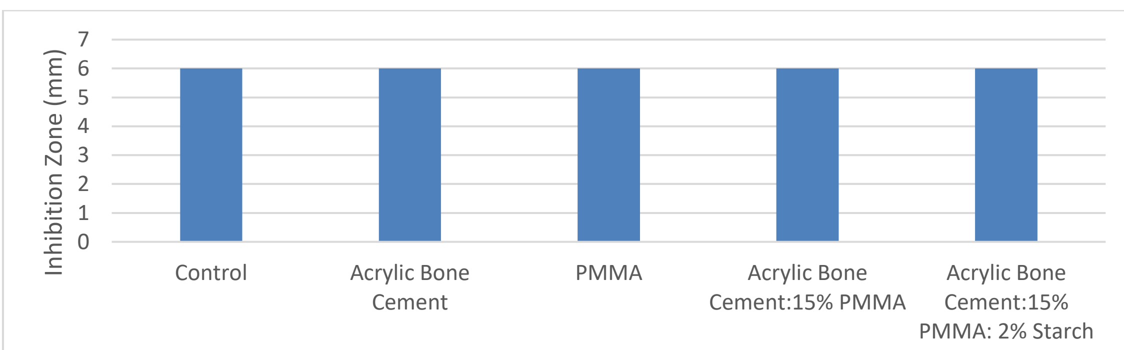
$$\%\text{Open Porosity} = \left(\frac{\text{Soaked Weight} - \text{Dry Weight}}{\text{Soaked Weight} - \text{Suspended Weight}}\right)*100 \quad .. [2]$$

Result and Discussion

Figures (2 and 3) shows, the biological activity of acrylic bone cement, PMMA, (Acrylic bone cement: 15% PMMA), and (Acrylic bone cement: 15 % PMMA: 2% starch) specimens, used in the current work against gram positive bacteria Bacillus subtilis and gram negative bacteria Escherichia coli respectively, all samples were kept during incubation periods at 37 ºC for 24 hours, the biological activity depend on inhibition zone diameter generated around each disk. So, it can be noted from this figure there is no inhibition zone (bacterial killed zone) around each sample after incubation for 24 hours as compared with control sample. Therefore, these samples can be utilized in bio applications as bone scaffold because these samples do not have biological effect on the bacteria microorganisms (13)

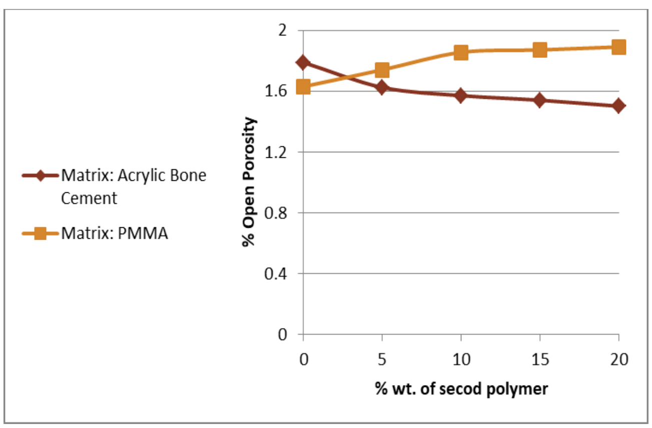


Figure, 2: Biological activity of acrylic bone cement, PMMA, (Acrylic bone cement: 15% PMMA), and (Acrylic bone cement: 15 % PMMA: 2% starch) against gram positive bacteria Baeillus *subtilis (B. subtilis)* . All samples were kept during incubation periods at 37 ºC for 24 hours.

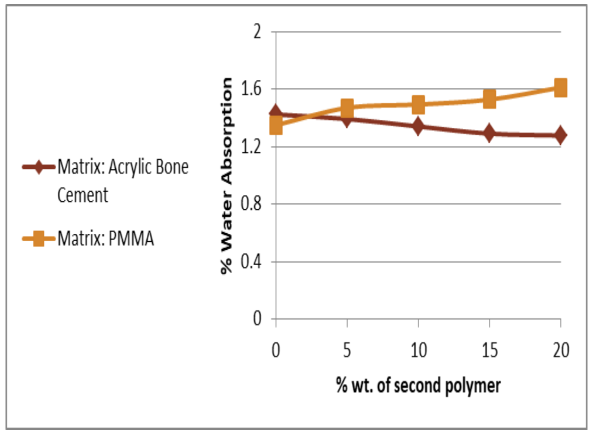


Figure, 3: Biological activity of acrylic bone cement, PMMA, (Acrylic bone cement: 15% PMMA), and (Acrylic bone cement: 15 % PMMA: 2% starch) against gram negative bacteria *Escherichia coli* (*E. coli*). All samples were kept during incubation periods at 37 ºC for 24 hours.

Figures (4, 5, 6 and 7) describe the behavior of open porosity and water absorption properties for all fabricated samples of polymeric blend and polymeric composites respectively. From these figures, it is observed, there is a significant difference between first group samples (Acrylic bone cement: % X PMMA), second group samples (PMMA: % X Acrylic bone cement), and third group samples (Acrylic bone cement: 15 % PMMA: % X Starch) as composite materials. It has been observed from figures (4 and 5), that the open porosity and water absorption of first group decrease with adding of second phase material in a matrix, as compared with their counterpart of the second group of polymeric blends. The reason behind such a behavior can be attributed to the strong bonding at the interfacial region (good wettability) between two phases of second group of polymeric blends which leads to decrease the micro voids and open spaces. Therefore, this reason leads to decreasing of the water absorption of the first group. However, higher water absorption of second group samples than a first group can be attributed to accumulate the dispersed phase, which increased the free volume and through the configuration of porosity and voids.



Figure, 4: Open porosity of (Acrylic bone cement: % X PMMA), and (PMMA: % X Acrylic bone cement) as a function of a second material content in blend



Figure, 5: Water absorption of (Acrylic bone cement: % X PMMA), and (PMMA: % X Acrylic bone cement) as a function of a second material content in blend.

The effect of addition of starch on the behavior of open porosity and water absorption properties can be observed from figures (6 and respectively, from these figures, it can be observed that the open porosity and water absorption increases with an increase in weight fraction of starch material. The increased in water absorption may be due to the hydrophilicity that's high-water sensitivity of starch [6]. The high-water uptake capability of the samples generates a rougher surface for cell adheres. In most reports starch material is subjected to reaction with methyl methacrylate to produce starch copolymer [14]. As well as, the open porosity increased with an increasing of starch content in the blend, this may be due to an increased in degradation rate of starch. Whereas, starch material is highly hydrophilic in nature due to it's a large number of hydroxyl groups on the backbones. One of major bottlenecks for the application of starch-based products is its great water absorption characteristic. More specifically, the high moisture absorption of starch deteriorates the adhesion with polymeric matrix and then lead to premature ageing by decomposition and loss of strength. It has been reported that the water absorption was strongly dependent on various factors, such as the immersion time, filler concentration, interfacial adhesion and the number of voids formed in the interfacial area. Additionally, it can be also seen that the water absorption and open porosity ratio was also increased as the weight percentage of starch increased (15).

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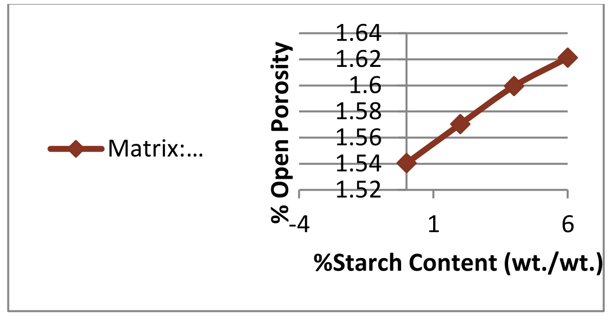
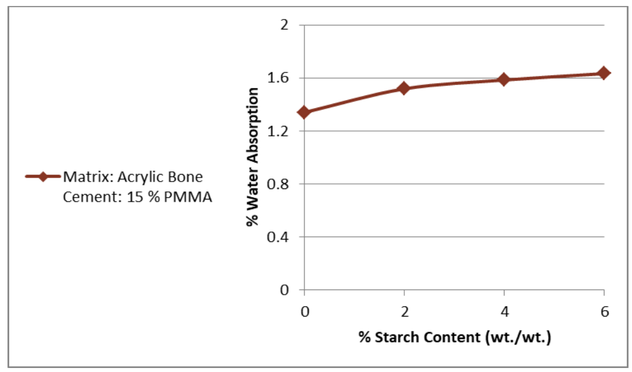


Figure, 6: Open porosity of (Acrylic bone cement: 15 % PMMA: % X starch) as a function of starch material content in blend.



Figure, 7: Water absorption of (Acrylic bone cement: 15 % PMMA: % X starch) as a function of starch material content in blend.

In the current work, efforts are made to develop PMMA polymeric composites materials which are utilized in the bone scaffold. The following conclusions were drawn based on the results of the tests received in the present work: The in vitro biological activity test showed that the all fabricated samples have no biological influence on the gram-positive bacteria Baeillus subtilis and gram-negative bacteria Escherichia coli. The results show that the first group of polymer blend have low open porosity and water uptake capacity than a second group. Open porosity and water absorption of third group increases with increasing starch content in a matrix. Based on the above, the samples of the third group (Acrylic bone cement: 15 % PMMA: % X starch) are promising substances in biochemical applications such as bone scaffold

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النشاط البيولوجي ، المسامية المفتوحة وامتصاص الماء للخليط البوليميري الثلاثي (بولي مثيل ميثا اكرياليت/ النشا) المستخدم في سقالة العظام

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الخلاصة

في هذه الدراسة تم تصنيع ثلاث مجاميع من النظام البيولوجي لسقالة العظم، تكونت المجموعة الاولى من) السمنت العظمي X % : بولي مثيل ميثا اكريلايت , (اما عينات المجموعة الثانية فتكونت من) بولي مثيل ميثا اكريلايت : % X السمنت العظمي (بنسب وزنية محددة من المادة الثانية و هي (5، 10، 15 ،20) في حين ان عينات المجموعة الثالثة تمثلت) السمنت العظمي : 15 % بولي مثيل ميثا اكريلايت X % : النشا (بنسب وزنية محددة من النشا و هي (2، 4، 6). تمت دراسة النشاط البيولوجي وامتصاص الماء في المختبر. أوضحت النتائج أن اختبار النشاط الحيوي في المختبر لجميع العينات المصنعة ليس لها تأثير بيولوجي على البكتيريا إيجابية الجرام (B. subtilis Bacillus subtilis) والبكتريا سالبة الجرام (E. coli) أظهرت النتائج أن المسامية المفتوحة وقدرة أمتصاص الماء للمجموعة الاولى هي أقل مما في المجموعة الثانية. وكذلك أدى تزايد الكسر الوزني للنشأ في المجموعة الثانية إلى زيادة المسامية المفتوحة وامتصاص الماء.

الكلمات المفتاحية: بولي مثيل ميثا اكريلايت ، النشا ، امتصاص الماء ، النشاط البيولوجي.