LAPORAN AKHIR TAHUN
PENELITIAN TERAPAN UNGGULAN PERGURUAN TINGGI
(PTUPT)

OPTIMASI BONE SCAFFOLD MAGNESIUM TERHADAP KEMAMPUAN OSTEOGENESIS SEBAGAI KANDIDAT ALVEOLAR BONE REPLACEMENT

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RINGKASAN

Augmentasi tulang alveolaris memerlukan bone graft atau scaffold. Saat ini bone graft atau scaffold yang beredar di pasaran dan sering digunakan dalam bentuk bubuk dan tidak memiliki kemampuan bearing loading (kemampuan menerima beban). Keberhasilan memperbaiki maupun meregenerasi tulang alveolaris merupakan tantangan saat ini. Hal ini disebabkan tulang alveolaris menyangkut sistem stomatognati yang kompleks dan menerima beban kunyah/oklusi. Penelitian ini bertujuan untuk mengembangkan biomimetic porous biodegradable scaffold yang memiliki kemampuan bearing loading untuk menggantikan cancellous alveolar bone.

Magnesium merupakan kandidat yang potensial sebagai biomimetic porous biodegradable scaffold. Magnesium memiliki sifat biocompatible maupun biodegradable dalam body fluid, dan memiliki mechanical properties yang hampir sama dengan tulang.
Stem cell mempunyai kemampuan untuk meningkatkan osteogenesis. Magnesium yang di seeding menggunakan Stem cells merupakan kombinasi ideal bagi scaffold yang digunakan pada tulang alveolaris.

Tujuan penelitian ini untuk mengembangkan proses manufacturing prototype solid free-form fabrication (SFF) porous magnesium scaffold untuk alveolar bone augmentation. Metoda fabrikasi memerlukan precise control untuk ukuran dan bentuk pore dan sehingga menyerupai architecture structure dari cancellous bone. Disain porous scaffold merupakan salah satu faktor penting dalam tissue engineering untuk menyerupai (mimicking) intrinsic extracellular environment.

Penelitian ini akan menghasilkan Bone scaffold yang memiliki kemampuan bearing loading merupakan konsep baru dari biodegradable metal untuk penggunaan klinis. Penelitian ini sejalan dengan Renstra dan peta jalan penelitian Universitas Airlangga, yang terletak pada bidang kesehatan dan obat terutama di bidang Stem Cell. Pemanfaatan Stem cells sejalan dengan Tema Riset 5 yaitu pengembangan Stem Cell dibidang Kedokteran Gigi sebagai bone augmentation. Disamping itu pemanfaatan sumber daya alam Indonesia sebagai material maju akan meningkatkan produktifitas dalam negeri akan menekan angka ketergantungan pada produk import, hal ini sesuai dengan Tema Riset 8 Universitas Airlangga.

Penelitian ini dilakukan selam 3 tahun :

1. Pada tahun pertama dilakukan uji properties compression pengaruh seeding Stem cells terhadap Magnesium bone scaffold menggunakan Instron.
2. Pada tahun kedua penelitian difokuskan pada studi efektivitas dari porous magnesium terhadap biodegradable pada animal models yang dianalisa menggunakan computational
3. Pada tahun ketiga penelitian difokuskan pada pemeriksaan optimasi Magnesium melalui pendekatan stem cell.

Penelitian ini didukung dengan team dari Faculty of Biosciences & Medical Engineering Universiti Teknologi Malaysia (UTM)-Johor Bahru yang mengembangkan penggunaan biodegradable metal dalam bidang ortopedi untuk penggunaan klinis. Dukungan diharapkan dapat mengakselerasi research sehingga akan mempercepat hasil akhir yang diharapkan yaitu Prototype dalam tiga tahun penelitian.

Pada tiap tahun akan dilakukan publikasi pada jurnal internasional terindex Scopus. Goal ultimate pada akhir tahun ketiga mendapat Prototype yang dapat diajukan paten untuk kepentingan komersial.

PRAKATA

Segala puji dan syukur peneliti panjatkan ke hadirat Tuhan YME, atas segala rahmat dan karuniaNya, sehingga peneliti dapat menyelesaikan penelitian ini dengan harapan dapat memberikan sumbangan pengetahuan tentang pemakaian bahan irigasi berbasis natural yang ramah lingkungan sehingga dapat menekan angka kegagalan perawatan saluran akar. Penelitian ini juga ditujukan untuk menekan angka ketergantungan terhadap produk impor dalam pelayanan kesehatan gigi sehingga dapat menekan biaya pelayanan kesehatan gigi. Harapannya masyarakat Indonesia mendapatkan pelayanan keehatan gigi dengan biaya yang lebih terjangkau.

Selama menyelesaikan penelitian ini, peneliti telah banyak memperoleh bimbingan, pengarahan dan bantuan, baik berupa ilmu pengetahuan maupun dukungan moril dari pemerintah Republik Indonesia melalui pemberian dana hibah PUPT. Dalam kesempatan ini, peneliti ingin menyampaikan rasa terima kasih yang sebesarbesarnya kepada :

1. Bapak Joko Widodo selaku Presiden Republik Indonesia
2. Prof. H. Mohamad Nasir, Ph.D., Ak sebagai Menteri di Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia yang telah memberi kesempatan kepada peneliti dalam menyelesaikan penelitian ini
3. Prof. Dr. Mohammad Nasih, SE., Mt., Ak., CMA sebagai Rektor Universitas Airlangga yang telah memberi kesempatan kepada peneliti untuk berkarya.

Peneliti menyadari bahwa penelitian ini masih banyak kekurangannya, namun mudah-mudahan penelitian ini bermanfaat bagi masyarakat Indonesia.

Surabaya, November 2018

Penulis

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BAB I
PENDAHULUAN

Augmentation merupakan prosedur tindakan medis untuk perbaikan atau penggantian volume tulang. Dalam bidang kedokteran gigi, penggunaan material maju dalam alveolar bone augmentation dilakukan pada kasus yang disebabkan oleh infeksi, dentoalveolar trauma, periodontal disease, traumatic extractions, disuese atrophy (aquired maupun congenital) serta pada beberapa kegagalan implant placement [Zheng et al,2014; Staiger et al, 2006]. Tindakan augmentasi pada tulang alveolar dapat dilakukan melalui Alveolar bone grafting/bone scaffold.

Material maju Bone scaffold yang saat ini beredar di pasaran pada umumnya berupa bubuk yang sulit digunakan pada kasus dimana dibutuhkan kemampuan menerima beban. Sedangkan bentuk metallic biomaterials seperti stainless steel, cobalt-chromium alloys, dan titanium alloys merupakan material yang sering digunakan bilamana diperlukan mechanical loading. Namun demikian selain memiliki mechanical strength yang tinggi dan fracture toughness yang baik, material tersebut juga memiliki kekurangan yaitu kemungkinan adanya pelepasa: ion toxic metallic serta kurangnya stimulasi terhadap pertumbuhan tulang baru dalam kaitannya dengan elastic moduli mismatch [Witte *et al*., 2008].

Kekurangan tersebut merupakan peluang research untuk mendapatkan material maju yang mampu mengeliminasi kekurangan tersebut dengan menggunakan biodegradable metals. Metal yang diharapkan memiliki kemampuan fungsi mekanis dan mempunyai kemampuan degradasi secara in vivo tanpa menyebabkan masalah toksikologi.

Bila dibandingkan dengan material yang berbahan dasar Fe dan Zinc Alloy yang terbaru, maka Magnesium dan Alloy-nya merupakan biodegradable metal yang potensial digunakan dalam biomedis [Gu et al,2009; Song and Atrens,1999]. Magnesium dan alloy-nya memiliki properti mekanik yang menyerupai tulang manusia; dengan; Young's modulus (41-45 GPa) maka Magnesium mirip dengan cortical bone (3–23 GPa) [Song and Atrens, 2003; Bigi et al,1993]. Disamping itu Magnesium memiliki densitas rendah dan rasio kekuatan terhadap berat yang cukup [Zreiqat et al.,2002; Zhang et al,2014].

Dari sudut pandang bioactivity, magnesium memiliki efek stimulasi pada pertumbuhan tulang dalam kaitannya terhadap pembentukan bone-apatite like hydroxyapatite crystals, yang sangat baik untuk kekuatan tulang [Geetha et al,2009; Gu et al,2010; Cheng et al,2016]. Mekanikal properti magnesium selanjutnya dapat dimanipulasi agar Young's modulus cancellous bone nya rendah (0.01–2.0 GPa), kemudian merubahnya kedalam struktur porous

sehingga sesuai dengan karakteristik cancellous bone [Rouwkema et al, 2008]. Struktur porous dari Magnesium akan menginduksi early vascularization menuju integrasi yang baik dengan regenerasi jaringan setelah degradasi gradual [Polo-Corrales et al,2014; Schaffler et al,2014]. Idealnya prosentase struktur porous berkisar 25-90% porositas dengan ukuran pore 10-1000μm yang merupakan kondisi yang ideal untuk infiltrasi nutrisi esensial, oksigen, dan sel progenitor bagi kehidupan sel [McNamara and Prendergast, 2007].

Alveolar Cancellous bone remodelling merupakan proses dinamis yang simultan terjadi melalui aksi osteoklas dan osteoblast pada proses pembentukan dan resorption tulang alveolaris. Osteosit telah diketahui sebagai orchestrator dalam proses alveolar bone remodelling yang menginisiasi isyarat dan pencetus pembentukan dan resorpsi pada tulang alveolaris [Schaffler et al,2014]. Mechanical loading yang berasal dari cyclic motion dari aktivitas fisiologis merupakan mekanisme pengatur yang akan merangsang mechano sensitif osteosit [McNamara and Prendergast, 2007]. Perubahan dalam stimulasi mekanik yang dihasilkan oleh mechanical stress melalui signal kimiawi yang dipicu oleh respon seluler memerintahkan alveolar bone modelling and remodelling [Schaffler et al,2014; McNamara and Prendergast, 2007].

Stem cell mempunyai kemampuan untuk meningkatkan osteogenesis. Magnesium yang di seeding menggunakan Stem cells merupakan kombinasi ideal bagi scaffold yang digunakan pada tulang alveolaris. Penelitian ini bertujuan untuk mengembangkan proses manufacturing solid free-form fabrication (SFF) porous magnesium scaffold sebagai material maju untuk alveolar bone replacement.

Disain porous scaffold merupakan salah satu faktor penting dalam tissue engineering menyerupai (mimicking) intrinsic extracellular environment untuk memberi ruang bagi Stem cells. Struktur porous memegang peranan penting dalam Stem sel untuk *attachment*, proliferasi, migrasi dan pertumbuhan jaringan. Disamping itu interkonektifitas *pore* juga memegang peranan penting dimana *complete pore interconnection* akan menyediakan jalur untuk biofluid dan pembentukan pembuluh darah.

Material maju Bone scaffold yang memiliki kemampuan bearing loading dengan sifat biodegradable merupakan konsep baru dari biodegradable metal untuk penggunaan klinis.

Penelitian ini didukung dengan team dari Faculty of Biosciences & Medical Engineering Universiti Teknologi Malaysia (UTM)-Johor Bahru yang mengembangkan penggunaan biodegradable metal dalam bidang ortopedi untuk penggunaan klinis. Dengan adanya dukungan diharapkan dapat mengakselerasi research sehingga akan mempercepat hasil akhir yang diharapkan yaitu Prototype dalam tiga tahun penelitian.

Pada tahun pertama dilakukan uji properties compression pengaruh seeding Stem cells terhadap Magnesium bone scaffold menggunakan Instron. Pada tahun kedua penelitian difokuskan pada studi efektivitas dari porous magnesium terhadap biodegradable pada animal models yang dianalisa menggunakan computational dengan micro-computed tomography (μCT) (Skyscan 1172; Kontich, Belgium). Rekonstruksi model menggunakan Amira 4 (Mercury Computer Systems, Inc. US) dan Materialise Mimics (Materialise, Belgium) kemudian dikonversi menggunakan (Comsol Multiphysics, USA). Pada tahun ketiga penelitian difokuskan pada pemeriksaan optimasi Magnesium melalui pendekatan stem cell.

Pada tiap akhir tahun akan dilakukan publikasi pada jurnal internasional terindex Scopus. Goal ultimate pada akhir tahun ketiga mendapat Prototype yang dapat diajukan paten untuk kepentingan komersial.

Tabel 1.1 Rencana Target Capaian Tahunan

No	Jenis luaran		Indikator Capaian		
			Th 1	Th 2	Th 3
1	Publikasi ilmiah	Internasional	published	published	Published
		Nasional terakreditasi	-	-	-
2	Pemakalah dalam temu ilmiah	Internasional	Sudah dilaksanakan	Sudah dilaksanakan	Sudah dilaksanakan
		Nasional	-	-	-
3	Invited speaker dalam temu ilmiah	Internasional	Sudah dilaksanakan	Sudah dilaksanakan	Sudah dilaksanakan
		Nasional	-	-	-
4	Visiting lecture	International	Sudah dilaksanakan	Sudah dilaksanakan	Sudah dilaksanakan
5	Hak Kekayaan Intelektual/ HKI	Paten	-	-	-
		Paten sederhana	-	-	terdaftar
		Hak cipta	-	-	-
		Merek dagang	-	-	-
		Rahasia dagang	-	-	-
		Desain produk industri	-	-	-
		Indikasi geografis	-	-	-
		Perlindungan varietas tanaman	-	-	-
		Perlindungan topografi sirkuit terpadu	-	-	-
6	Teknologi tepat guna		-	-	-
7	Model/purwarupa/desain/karya seni/rekayasa sosial		-	-	-
8	Buku ajar/ISBN		Tidak ada	Draft	Proses editing
9	Tingkat kesiapan teknologi		4	5	6

BAB II
TINJAUAN PUSTAKA

2.1 RENSTRA dan ROAD MAP Perguruan Tinggi

Universitas Airlangga adalah tempat menumbuh-kembangkan budaya penelitian di perguruan tinggi dan mendorong pendayagunaan hasilnya guna mendukung misi pendidikan tinggi dan pembangunan nasional secara berkelanjutan; tempat pengamalan IPTEKS berdasarkan kebutuhan masyarakat oleh Universitas Airlangga, langsung ke masyarakat luas, melembaga, profesional melalui metode ilmiah. Kegiatan penelitian dan inovasi di Universitas Airlangga diklasifikasikan ke dalam tiga program, yaitu (1) Program Pembinaan, (2) Program Penelitian Mandiri dan (3) Program Penelitian yang terkait dengan Kebijakan Publik. Program pembinaan diarahkan untuk menghasilkan penelitian dan, publikasi bertaraf nasional/internasional, bahan/materi pendidikan dan bimbingan untuk program S1/S2/S3, dan peningkatan budaya meneliti dan mengabdi kepada masyarakat yang produktif. Program ini mencakup Penelitian dan Inovasi yang didanai Ditlitabmas Ditjen Dikti termasuk Penelitian Unggulan Perguruan Tinggi.

Sesuai dengan RIP (Rencana Induk Penelitian) Universitas Airlangga, maka kegiatan penelitian lebih diarahkan untuk menciptakan inovasi dan pengembangan IPTEKS. Penelitian diarahkan dan dikembangkan untuk penguatan penelitian dasar, inovatif dan terapan. Pengembangan penelitian diarahkan untuk menciptakan unggulan yang menjadi ciri khas Universitas yang tercermin dalam roadmap penelitian untuk memenuhi kebutuhan nasional dan internasional. Tema riset unggulan Universitas Airlangga meliputi:

No	Bidang/Fak	Tema Riset Unggulan
1	Pertanian	1. Pemberdayaan masyarakat pesisir dan tenteral 2. Ketahanan pangan
2	Kesehatan/Farmasi	3. Pengembangan obat bahan alam 4. Kanker dan Autoimun 5. Penanggulangan penyakit tropis 6. Pengembangan Stem cell
3	Sosial ekonomi dan hukum	7. Sistem pengelolaan layanan kesehatan penduduk miskin 8. Pengembangan regulasi dan model kebijakan 9. Pemilu dan demokrasi
4	Matematika dan ilmu pengetahuan alam	10. Pengembangan material maju 11. Produksi tanaman transgenik 12. Produk hasil fermentasi mikro-organisme 13. Bioremediasi lingkungan dan pengelolaan limbah 14. Pemodelan di bidang life science, ekonomi dan industri berbasis ict
5	Psikologi dan budaya	15. Integrasi dan Harmonisasi Nasional 16. Seni dan budaya untuk menunjang industri kreatif 17. Pembangunan manusia dan daya saing bangsa

Gambar 2.1 Tema riset unggulan Universitas Airlangga

Sesuai dengan tujuan penelitian ini yaitu untuk mendapatkan material maju bone augmentation melalui bone regeneration dengan menggunakan Magnesium bone scaffold yang di optimasi dengan Chitosan maka penelitian ini selaras dengan bidang pengembangan Stem Cell dan pengembangan material maju di bidang kesehatan.

TEMA RISET 5 : PENGEMBANGAN STEM CELL

Kompetensi/keahlian	Isu-Isu strategis	Konsep pemikiran	Pemecahan masalah	Topik Penelitian Fakultas
Natural Science Ilmu Farmasi Ilmu Kedokteran Ilmu Kedokteran Hewan	Eksplorasi dan pengembangan teknologi stem cell di Indonesia yang minim aplikasi teknologi stem cell pada subje manusi yang masih bolum jelas pada aspeketik, efikas dan keamanan	Pegembangan metode alternative dalam menyelesaikan masalah kondisi terminal penyakit keganasan, degenerative imunologi, traumatology dan kesehatan reproduksi	Pengembangan penatalaksanaan keganasan, penakit degenratif, traumatlogi, imunologi dan kesehatan reproduksi berbasis stem cell	1 Stem cell pada terapi keganasan stem cell pada terapi penyakit degenaritif stem cell pada traumatologi stem cell pada penyakit imunologi stem cell pada kesehatan reproduksi kajian etik penerapan stem cell di manusia

(RIP Universitas Airlangga)

TEMA RISET 5 : PENGEMBANGAN STEM CELL

Topic penelitian	Tahun				
	2016	2017	2018	2019	2020
Stemcells untuk dental pulp caping material	Isolasi stem cells pulpa Karakterisasi proliferasi dan stemcells pulpa	Uji differensiasi	Uji Pre Klinik untuk pulp caping	Uji Klinik stemcells pulpa sebagai bahan pulp caping bone regeneration dan bone augmentation dan periodontal regeneration	
Penggunaan Bahan alam /Chitosan sebagai scaffold	Uji biocompatibility dan uji toxiciti	Uji Pre klinik	Uji Klinik		

(RIP Universitas Airlangga)

TEMA RISET 8 : PENGEMBANGAN MATERIAL MAJU DI BIDANG KESEHATAN

Kompetensi/ Keahlian	Isu-Isu Strategis	Konsep Pemikiran	Pemecahan Masalah	Topik Penelitian Fakultas
Ilmu Farmasi Natural Science Ilmu Kedokteran Gigi	(A) Belum optimalnya ketersediaan pemerataan dan keterjangkauan sediaan farmaka kosmetik dan alat kesehatan Sebagian besar bahan baku sediaan farmaka kosmetik dan alat kesehatan ini masih diimpor sedangkan penggalian potensi sumberdaya alam lokal sangat terbatas	(A) Pengembangan Material Maju Mendukung Teknologi Kesehatan Obat	(a) Pengembangan Bahan Baku Obat dan kosmetik (b) pengembangan obat bahan alam (c) pengembangan alat kesehatan dan kedokteran berbasis nanoteknologi (d) Desain material smart implant Pengembangan material	(1) Pengembangan material berukuran nanometer untuk bahan baku kosmetik dan obat (2)Pengembangan biomaterial untuk tulang gigi buatan/ *artificial organ* lain (3)Pengembangan material biopolimer untuk pembuatan produk kantong darah, katup jantung buatan, dsb

	(B) Isu *global warming* dan *alternative energy* menuntut adanya pembaharuan dalam pengembangan material untuk aplikasi transportasi ke depan	(B) Pengembangan Material Maju Mendukung Teknologi dan Manajemen Transportasi	maju cerdik (*smart advanced materials*),baik struktural maupun fungsional, yang mampu memberikan performa/ keunggulan yang responsif bila berada di lingkungan yang kondusif dengan dukungan nanoteknologi	(1) Pengembangan material untuk mengurangi gas emisi dari transportasi (2) Pengembangan Bahan *Nano-Coating* Ramah Lingkungan

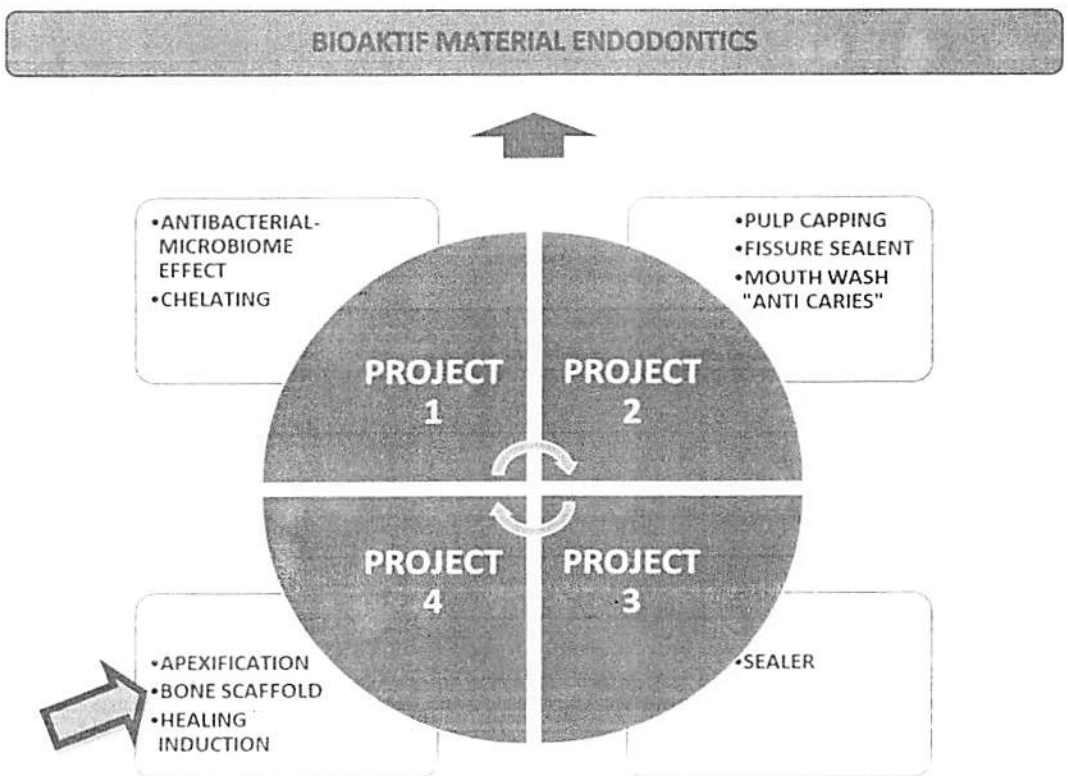
(RIP Universitas Airlangga)

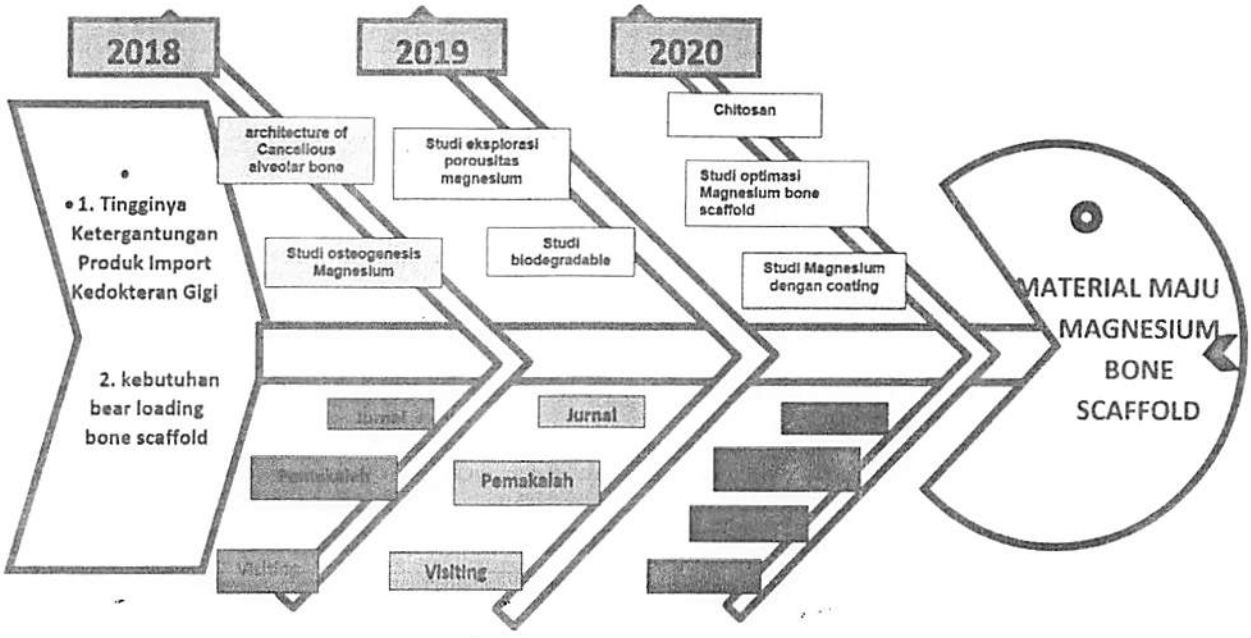
TEMA RISET 8 : PENGEMBANGAN MATERIAL MAJU DI BIDANG KESEHATAN

Topic penelitian	Tahun				
	2016	2017	2018	2019	2020
Pengembangan biomaterial untuk tulang/ gigi buatan/ *artificial organ* lain	Identifikasi struktur Biomaterial tulang dan gigi	Uji struktur material yang sesuai tulang dan gigi	Penetapan struktur tulang dan gigi dari bahan material artificial	Pembuatan prototype tulang dan gigi buatan	Uji tulang dan gigi buatan dalam kultur sel
	Rekayasa material inplant berbah	Uji in-vitro	Uji in-vivo	Pembuatan prototype sesuai yang dibutuhkan	Aplikasi material implant

(RIP Universitas Airlangga)

2.2 Roadmap peneliti




2018
2019
2020
• 1. Tingginya Ketergantungan Produk Import Kedokteran Gigi
2. kebutuhan bear loading bone scaffold
architecture of Cancellious alveolar bone
Studi osteogenesis Magnesium
Studi eksplorasi porousitas magnesium
Studi biodegradable
Chitosan
Studi optimasi Magnesium bone scaffold
Studi Magnesium dengan coating
Jurnal
Pemakalah
Visiting
MATERIAL MAJU MAGNESIUM BONE SCAFFOLD

7

OUTPUT

Penelitian ini diharapkan dapat menghasilkan material maju yang dapat digunakan dalam perawatan bidang kedokteran khusunya kedokteran gigi sehingga bisa mengurangi ketergantungan produk import berupa material maju sebagai kandidat alveolar bone replacement. Penelitian ini juga diharapkan dapat berkontribusi dalam memanfaatkan limbah perikanan untuk digunakan di bidang pengembangan Stem Cell dalam kedokteran gigi.

OUTCOME

Pengurangan produk import akan menekan biaya perawatan gigi. Masyarakat Indonesia akan mendapatkan pelayanan kesehatan gigi dengan biaya yang lebih terjangkau. Penemuan dan penggunaan material maju dalam perawatan di bidang Kedokteran Gigi akan meningkatkan keberhasilan perawatan alveolar bone replacement.

BAB III
TUJUAN DAN MANFAAT PENELITIAN

3.1 Tujuan

Tujuan Umum

Penelitian ini bertujuan untuk meningkatkan Optimasi Bone Scaffold Magnesium Terhadap Kemampuan Osteogenesis Sebagai Kandidat Alveolar Bone Replacement

3.2 Tujuan Khusus

Untuk mengetahui bagaimana pengaruh properties compression Magnesium bone scaffold terhadap pemberian seeding Stem cells.

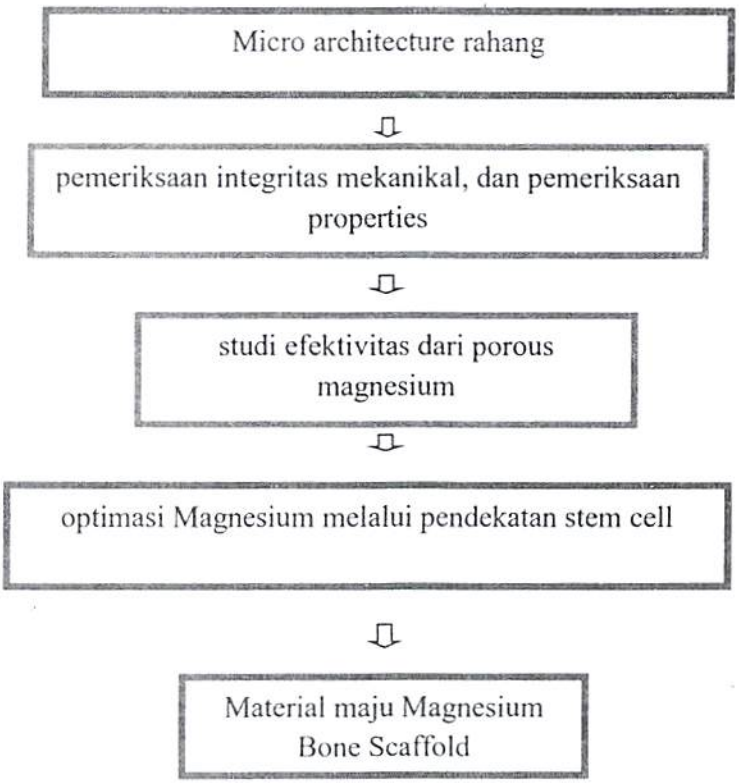
3.3 Manfaat

Penelitian ini diharapkan dapat memberikan informasi ilmiah tentang potensi Bone Scaffold Magnesium Terhadap Kemampuan Osteogenesis Sebagai Kandidat Alveolar Bone Replacement.

BAB IV

METODE PENELITIAN

4.1 Research mapping

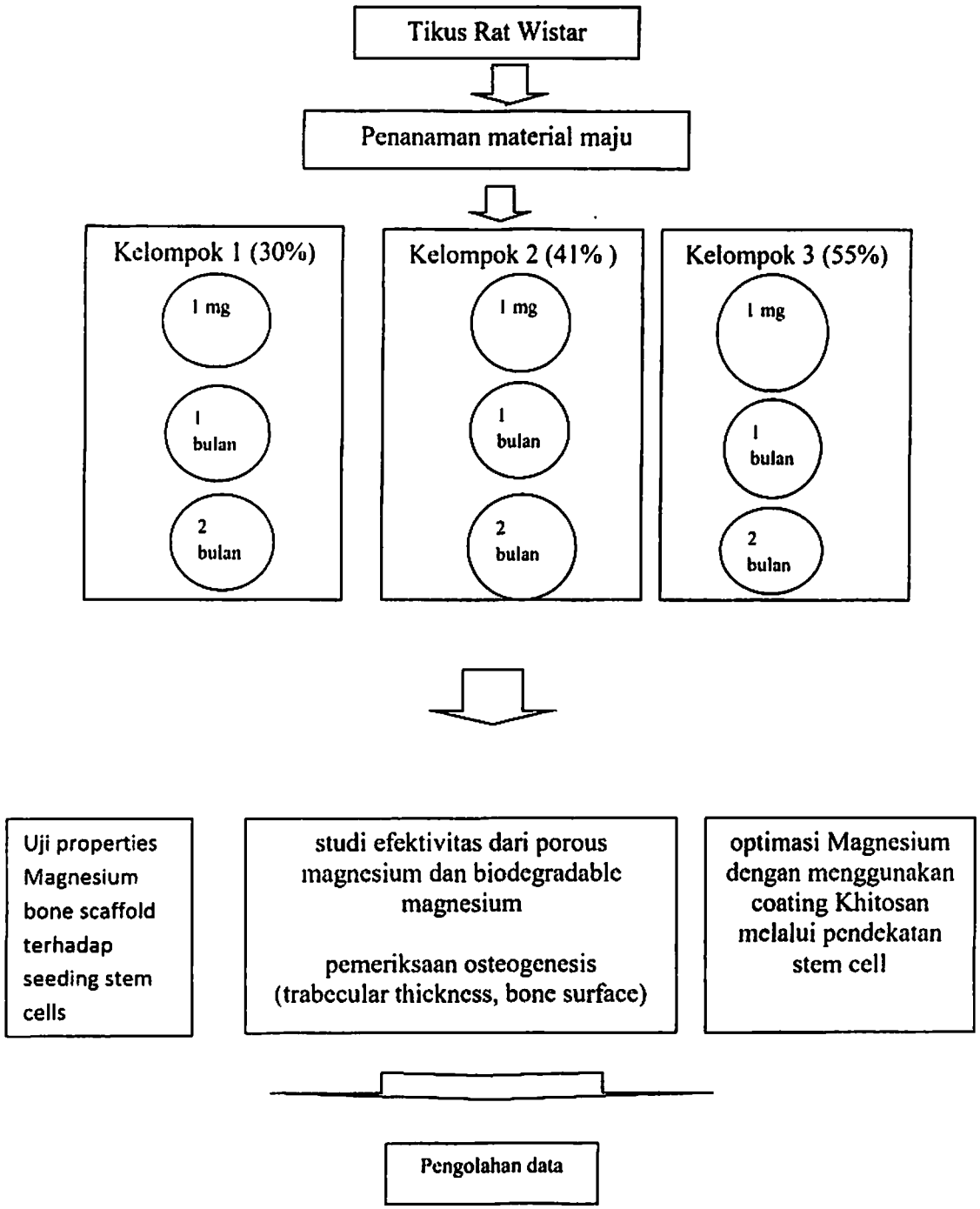


Keterangan:

: sudah dilakukan

: penelitian selanjutnya

4.2 Alur penelitian



4.3 Sampel :

Tikus Rat Wistar yang telah ditanam material maju Magnesium bone scaffold

4.4 Lokasi Penelitian

Dilakukan di laboratorium Fakultas Kedokteran Hewan, Universitas Airlangga, Surabaya dan Faculty of Bioscience and Medical Engineering, UTM, Malaysia.

4.5 Cara Kerja

Material dan metoda

Persiapan material maju Magnesium bone scaffold

Pure magnesium rod dengan diameter 25.4 mm dan kemurnian 99.9% (Goodfellow Inc, Cambridge, UK) dipotong menjadi spesimen dengan ukuran diameter 5mm dan kedalaman 5 mm. Inter-connected holes pada spesimen dengan porositas yang bervariasi buatan pabrik menggunakan teknik solid free form. Pada permukaan spesimen dilakukan grinding dan polishing kemudian dibersihkan menggunakan ultrasonik.

Hewan coba dan prosedur pembedahan

Dua puluh satu tikus Rat Wistar dalam 3 group. Setiap group terdiri dari 7 tikus. Setiap grup mendapatkan persentase porousitas magnesium scaffold yang berbeda. Dilakukan anestesi pada hewan coba dengan menggunakan Ketamin (25 mg/kg) melalui intravena. Prosedur pembedahan: dilakukan insisi pada regio frontal pipi kanan. Kemudian dengan menggunakan mata bur silinder dilakukan pembuatan defek pada tulang alveolaris sesuai dengan ukuran Magnesium bone scaffold. Setelah itu dilakukan pemasangan scaffold pada daerah defek dengan cara minimal invasif kemudian dilakukan penutupan.

Gambar 4.1 lokasi pemasangan bone scaffold gold standar untuk model kerusakan pada tulang alveolaris (Is bone transplantation the gold standard for repair of alveolar bone defects?. Raposo *et al*., 2014)

μCT Assay

Post implantasi, pada termin ke- 1 minggu, 1 bulan, dan 2 bulan, semua subyek penelitian di scan menggunakan μCT. Setiap sisi bone scaffold yang ditanam dilakukan scanning dengan ketebalan irisan slice 36 μm. Ukuran voxel adalah 36×36×36 μm3. Volume yang diinginkan disleksi menggunakan metoda semiautomatic contouring. Scanning yang dihasilkan merupakan fitur seri planar transverse berwarna abu-abu. Semua fitur akan di segmentasi menggunakan low-pass filter untuk membuang noise, dan dengan threshold yang sama untuk mengekstrak fase tulang. Dengan software dapat digunakan untuk memproses parameter-parameter struktural, sedangkan untuk menganalisa jumlah tulang baru yang terbentuk, digunakan area yang berada di radius 3 mm dari bone scaffold yang ditanam. Fraksi volume tulang (Bone volume/Total volume, BV/TV), ketebalan trabekula (Tb. Th) dan jumlah trabekula (Tb. N) tulang baru dianalisa kemudian laju degradasi dari bone scaffold dideterminasi

Integritas Mekanikal

Bentukan jaringan baru disekeliling bone scaffold merupakan inti dari evaluasi mechanical strength. Pada subyek spesimen dilakukan compression test menggunakan mesin universal testing (The FastTrack 8874, Instron, Norwood, USA) dengan strain rate 0.005/s dan beban 25 kN.

BAB V

HASIL DAN LUARAN YANG DICAPAI

HASIL

5.1 Degradasi porous Mg

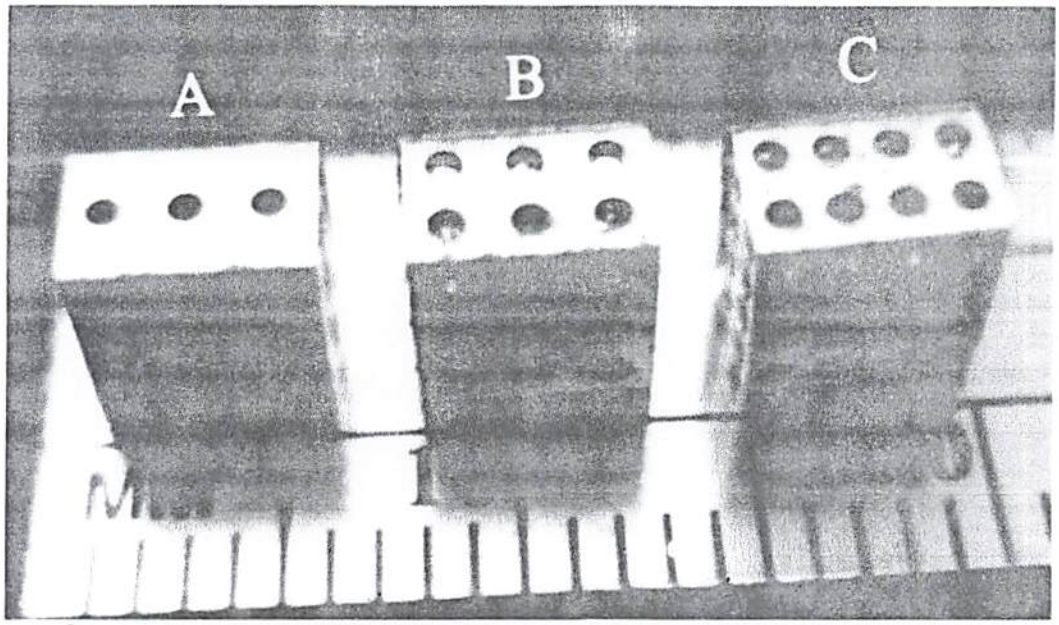


Figure 1 The three types of porous Mg specimens used in this study

Table 1 Overview of the detailed geometries for the porous Mg specimens (Md Saad et al 2016, 2017)

Type	Porosity	Surface area	Volume	Mass per Surface area
A	30%	189.30 mm²	52.57 mm³	0.44 kg m⁻²
B	41%	209.81 mm²	44.57 mm³	0.34 kg m⁻²
C	55%	225.75 mm²	33.83 mm³	0.24 kg m⁻²

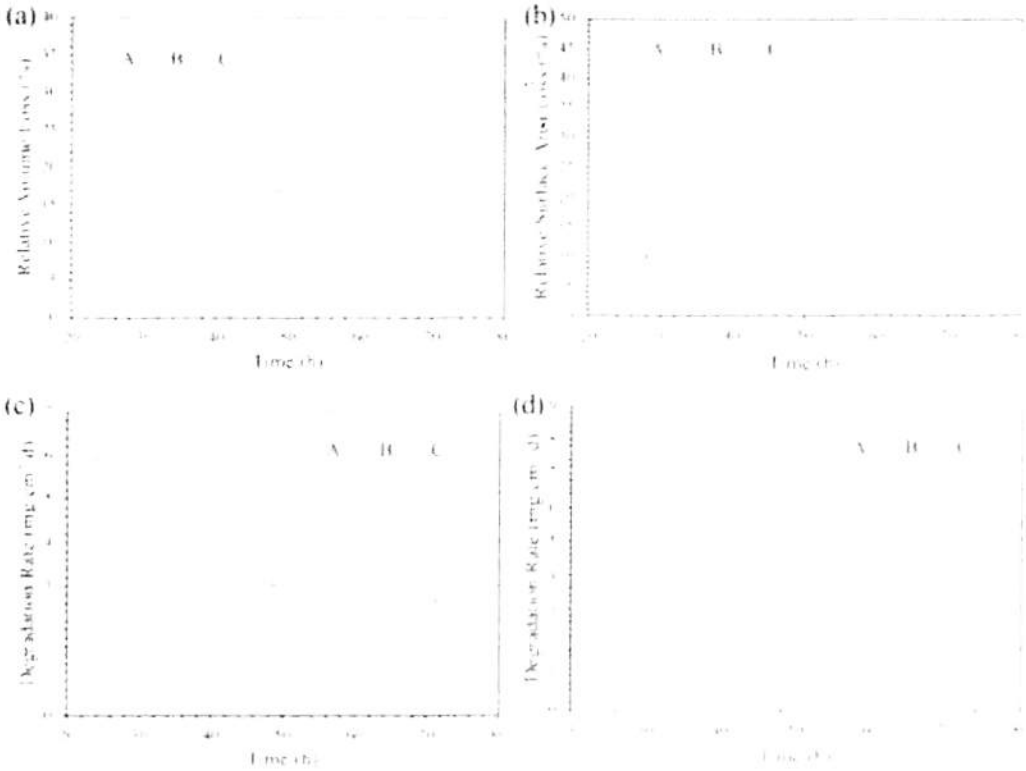


Figure 6 Degradation characterisation. (a) relative volume loss (%), (b) relative surface area loss (%), (c) degradation rate of specimens using original surface area, and (d) degradation rate of specimens using degraded surface area.

5.2 Karakterisasi morfologi

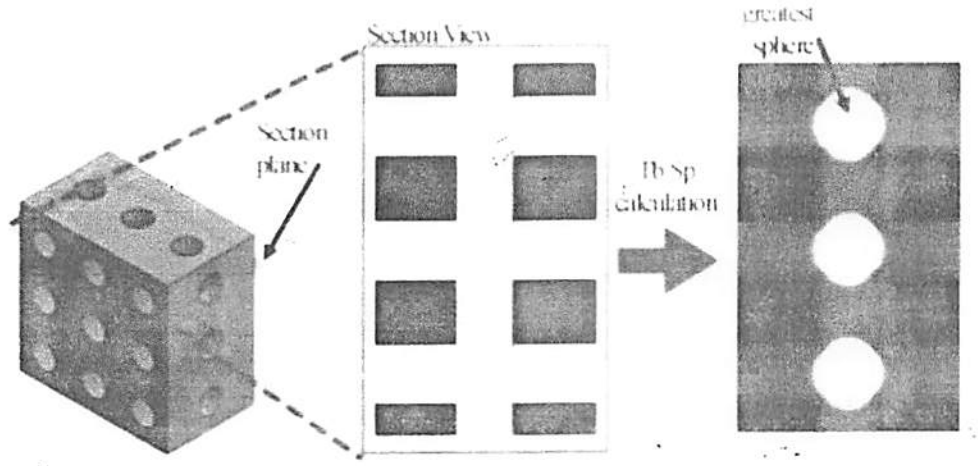


Figure 2 Trabecular separation (Tb.Sp) of Specimen A prior to the immersion test

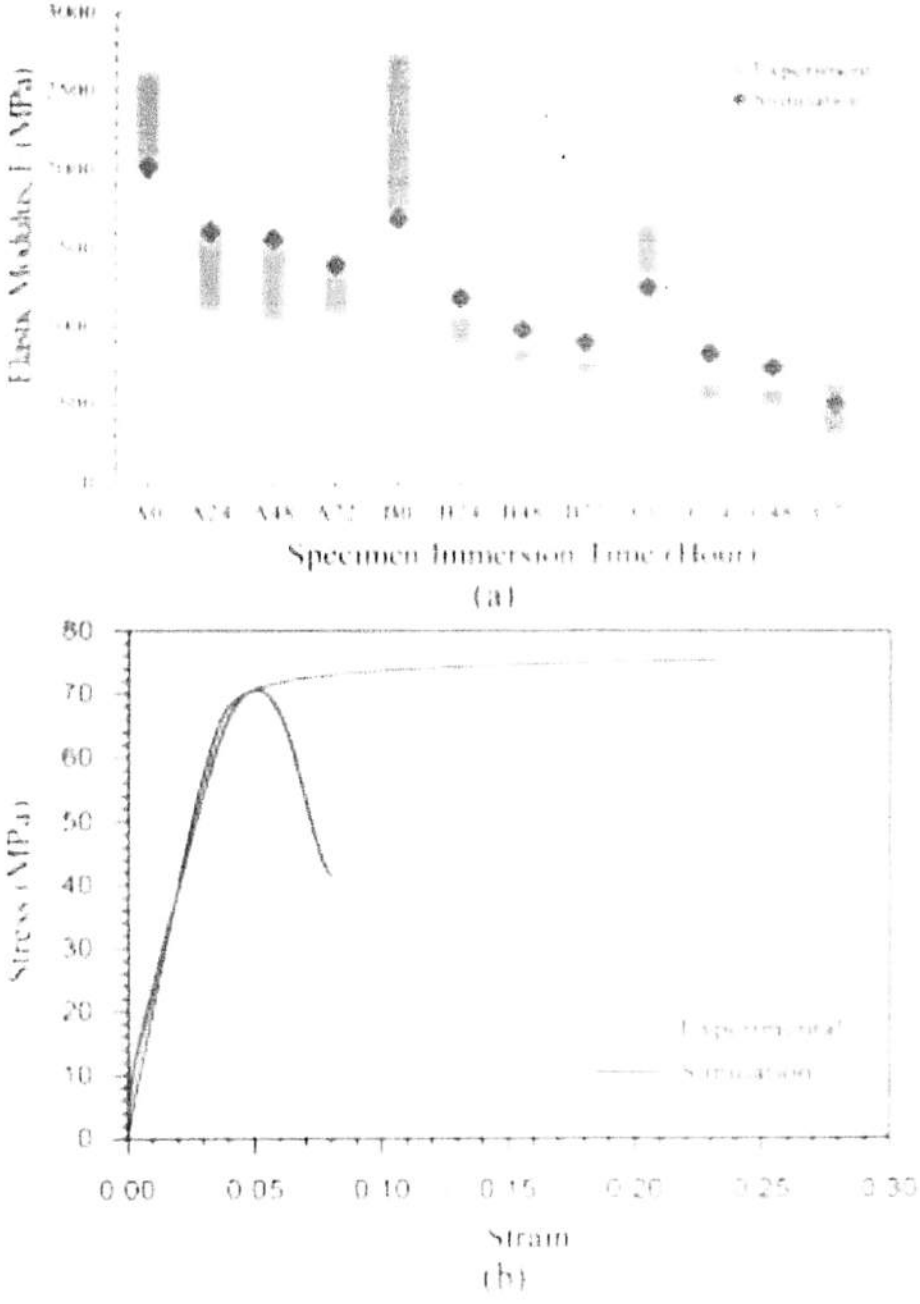


Figure 7 (a) Elastic modulus of the specimens determined by means of experiment and simulation (Md Saad et al. 2016) and (b) Comparison of compressive stress-strain curves between the experimental and finite element simulations for Specimen A prior to the immersion test (A0)

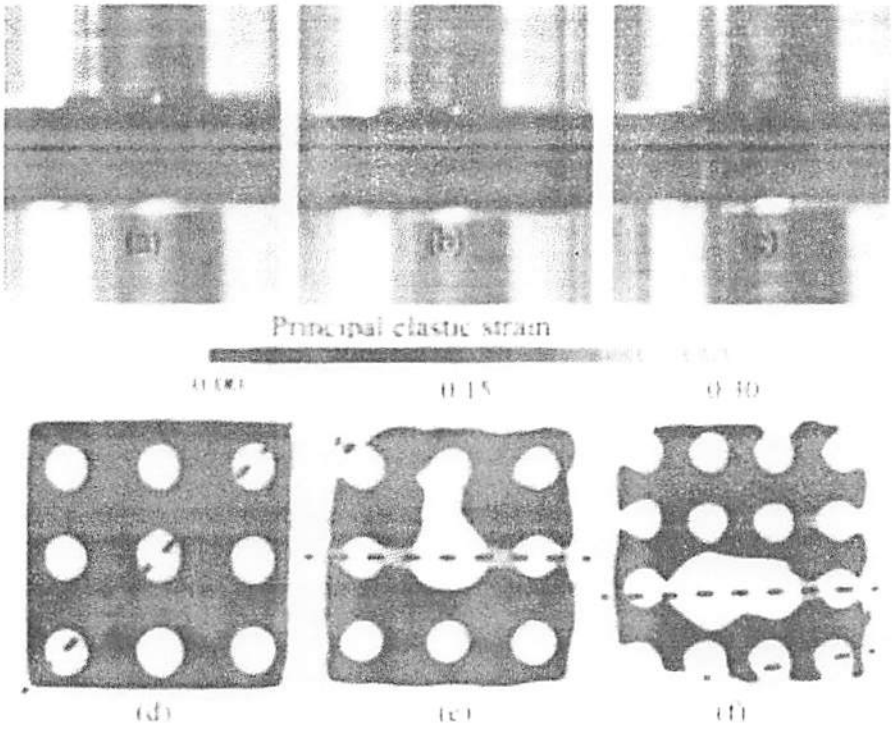


Figure 8 Contour plots of the fracture patterns after the dynamic immersion test (a) Specimen A, (b) Specimen B, and (c) Specimen C, and principal plastic strain contours from FEA (d) Specimen A, (e) Specimen B, and (f) Specimen C

5.3 Teknik segementasi

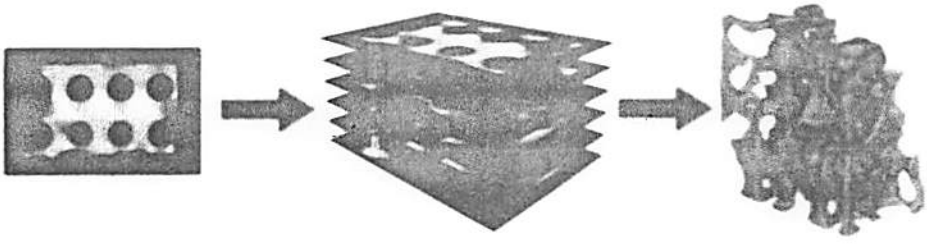


Figure 3 3D reconstruction of Specimen C (percentage porosity of 55%) from micro-CT images using Mimics software after 72 h of immersion time

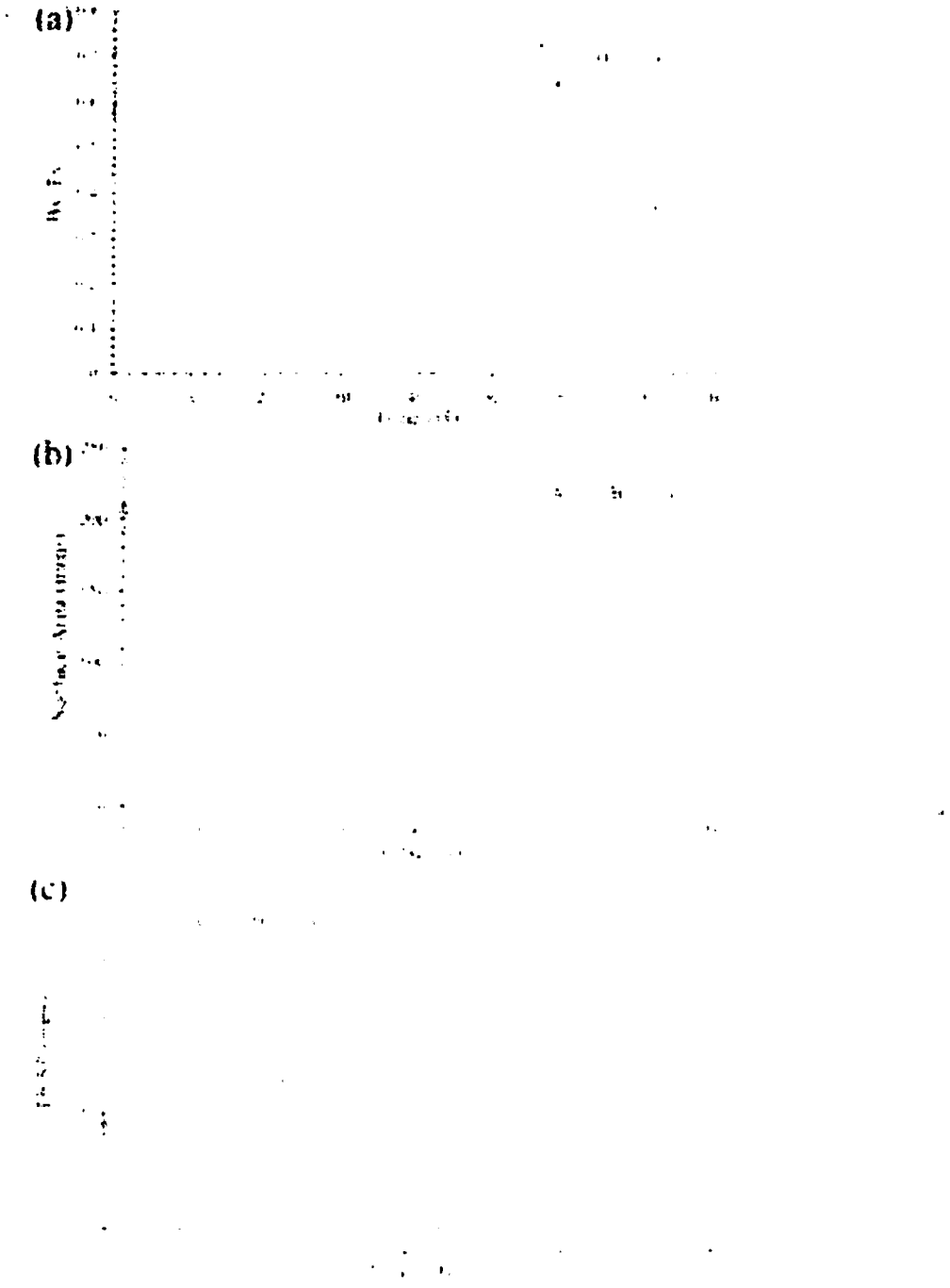


Figure 9 Morphological changes in porous Mg under dynamic degradation test (a) volume fraction (Bv/Tv), (b) surface area, and (c) trabecular seperation (Tb.Sp)

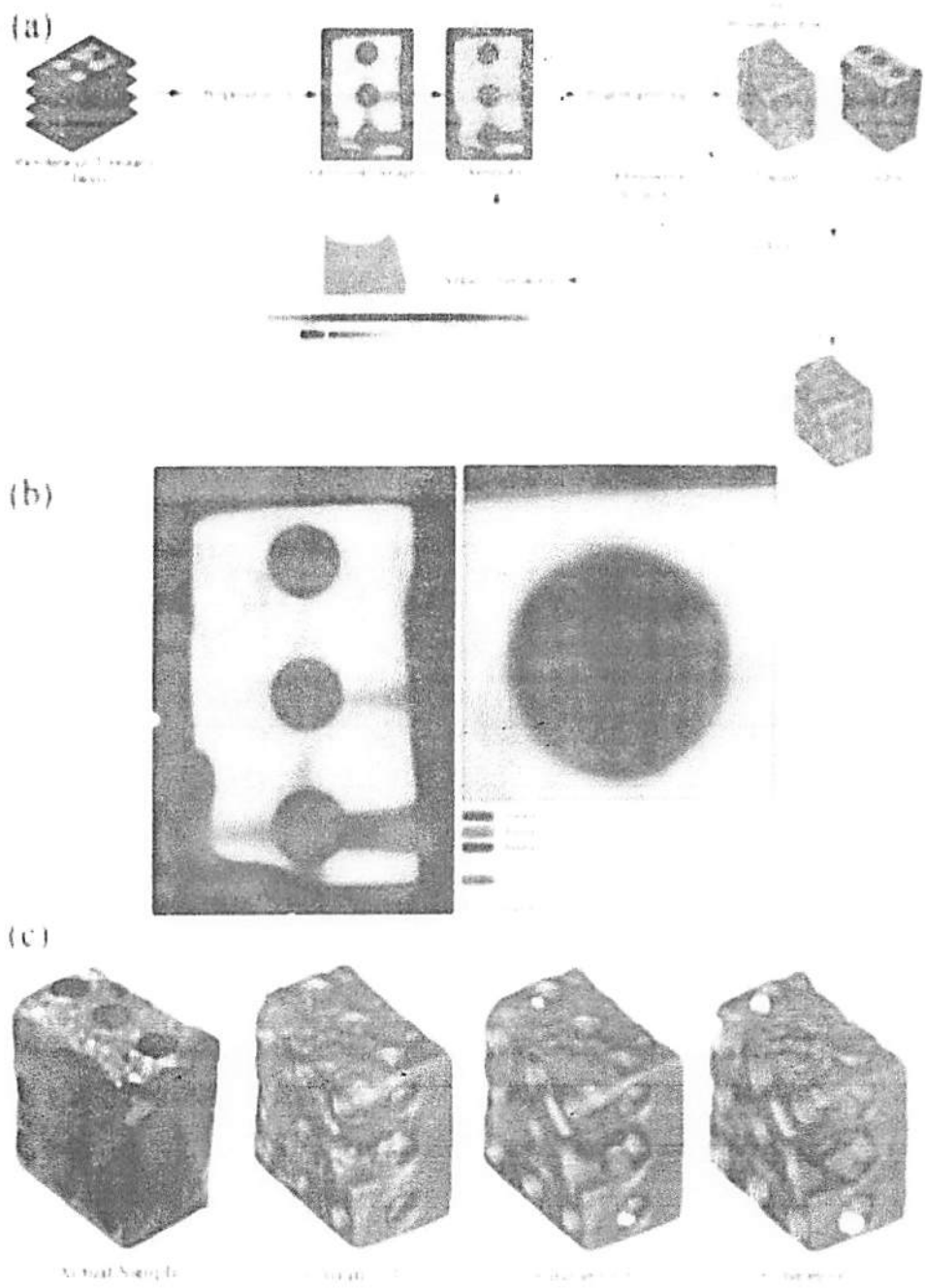


Figure 4 Iterative segmentation. (a) Flowchart of the iterative segmentation based on thresholding optimisation, (b) An example of the scaffold model segmentation based on the iterative thresholding method in Mimics software, and (c) A comparison of the 3D models from the iterative threshold

Table 2 Summary of the virtual model created with manual iterative threshold segmentation

Iterative	Threshold Values	Volume of Mimics model (mm3)	Actual volume mm3)	Percentage Error (%)
=1	-592	50 59	39 89	26 82
=2	-40	45 32	39 89	16 13
=3	0	41 61	39 89	4 33
=4	113	40 57	39 89	1 72
=5	150	40 19	39 89	0 75
=6	186	39 87	39 89	0 03
=7	200	39 73	39 89	0 39
=8	240	39 41	39 89	1 19
=9	267	39 16	39 89	1 82
=10	300	38 89	39 89	2 49
=11	1076	32 53	39 89	13 53
=12	1416	28 83	39 89	27 72

LUARAN YANG DICAPAI:

1. Paper tersebut telah di accepted pada Jurnal Internasional ter index Scopus Q1 (Annals of Biomedical Engineering Journal)
2. Telah di presentasikan pada International Conference : World Endodontic Conference (IFEA) di Seoul, Korea Selatan
3. Draft paten yang akan didaftarkan pada tahun ke 2 (2019)

BAB VI
RENCANA TAHAPAN BERIKUTNYA

Pada tahun berikutnya akan dilakukan uji Fatique

BAB VII
KESIMPULAN DAN SARAN

6.1 Simpulan

Dari hasil penelitian yang telah dilakukan, dapat disimpulkan bahwa:

Berdasarkan hasil pemeriksaan dengan menggunakan µCT dan dianalisa menggunakan FEA bahwa hubungan antara struktural properties dengan parameter morfologi dapat diidentifikasi dengan signifikan.

1. Persentase *volume loss* secara komputasional dapat digunakan dan berhubungan dengan *mass loss*
2. Degradation rate dideterminasi dengan menggunakan degradasi permukaan pada area yang terendam dengan laju kecepatan 20% lebih tinggi disbanding dengan degradation rate yang diperoleh menggunakan area permukaan *original*
3. Dynamic degradation secara signifikan berhubungan dengan perubahan morfologi porous MG seperti volume fraction, surface area, dan trabekula dengan nilai <0,005

6.2 Saran

Berdasarkan hasil penelitian diatas maka saran yang dapat disampaikan adanya penelitian lebih lanjut tentang uji fatique .

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Lampiran 1

MANUSCRIPT

Impacts of dynamic degradation on the morphological and mechanical characterisation of porous magnesium scaffold

Keyword: Dynamic immersion test; Finite Element Analyses; Dynamic Degradation; Porous Magnesium; Morphological Parameters

Abstract

This study employs a computational approach to analyse the impact of morphological changes on the structural properties of biodegradable porous Mg subjected to a dynamic immersion test for its application as a bone scaffold. Porous Mg was immersed in a dynamic immersion test for 24, 48, and 72 hours. Twelve specimens were prepared and scanned using micro-CT and then reconstructed into a 3D model for Finite Element Analysis. The structural properties from the numerical simulation were then compared to the experimental values. Correlations between morphological parameters and structural properties and fracture type were then made. The relative losses were observed to be in agreement with relative mass loss done experimentally. The degradation rates determined using exact (degraded) surface area at particular immersion times were on average 20% higher compared to the degradation rate obtained using original surface area. The dynamic degradation of this material is shown to be significantly impactful, as observed from the morphological changes in volume fraction, surface area, and trabecular separation, which in turn affect structural properties, as immersion time increases.

1.0 Introduction

Bone scaffolds made of biodegradable metal for cancellous bone replacement has been shown to be promising scaffolds for triggering new bone matrices and are good for load bearing purposes while the bone is healing. The degradation of biodegradable metal can be determined extensively by means of weight loss measurement and electrochemical analyses, both of which are *in vitro* test measurements. However, it is very difficult to measure the degradation of biodegradable metal inside animal bones using *in vivo* measurement. Therefore, for animal testing measurement, degradation is often determined using computed tomography (CT), which also has other uses besides determining degradation. Meanwhile, from the CT data set, parameters such as the morphological and structural properties of the remaining degraded material can be analysed using an image analyser and Finite Element Analysis (FEA), respectively. At the same time, new tissue that grows while the bone is healing can also be closely monitored. Cancellous bone acts as the host tissue once the scaffold is implanted. It possesses a complex microarchitecture, which plays a major role in determining cancellous bone properties and bone quality (Judex et al. 2003; Shi et al. 2009). In order to effectively integrate bone scaffold with the host tissue, the physical characteristics of the scaffold such as porosity and mechanical properties should be similar to that of cancellous bone (Bose et al. 2012). In addition, controlled degradation rate of the materials—as structural supports—at the earliest stage of bone healing is critically important (Zheng et al. 2014).

Biodegradable metals such as magnesium and its alloys have fascinating features that make them suitable as bone scaffolds. Mg has the potential to degrade *in vivo* without causing toxicological problems (Zheng et al. 2014). Besides that, it induces stimulatory effects in bone growth due to the formation of bone-apatites such as hydroxyapatite crystals (Bigi et al. 1993) and possesses mechanical properties similar to human cancellous bone (Md. Saad et al. 2016). Therefore, to track the physical changes in porous magnesium under a dynamic degradation environment, the degradation rate and morphology were analysed using CT measurement data sets and the structural properties determined using FEA.

Cancellous bone is a key structural support in load bearing applications. Besides supporting more than 75% of the total body weight (Liu et al. 2009), it also acts as a counter load through the cortical bone structure. The proficient microarchitecture of a cancellous bone structure significantly functions as an absorber upon mechanical loading. Mechanical loads from physiological activities induce tensile, compressive, and shear stresses on the cortical bone. These are adjusted by the bore of the cancellous bone (Liu et al. 2009). These stresses are important for triggering the bone remodelling process to provide bone strength. The mechanical properties of the material such as modulus of elasticity, and compressive and tensile strength are also of import. These properties are highly dependent on viscoelasticity (depending on the load applied) and anisotropy (depending on load orientation) (Helgason et al. 2008). The relationship between the morphological and structural properties of cancellous bone was measured via experimental works as well as computational methods (Syahrom et al. 2011). The structural properties have been widely assessed through compression tests in experimental works; however, acquiring the morphological information of materials by means of experimental works is difficult and almost impossible (Sulong et al. 2016). Computational methods are much more preferred, as they provide the morphological details of materials up to the micro-level, especially for irregular or complex porous structures.

Bone marrow located inside the porous structure of cancellous bone is subjected to mechanical signals transmitted via canaliculi networks through load bearing demand as a result of routine physical activity (Metzger et al. 2015). Bone marrow is a complex and highly vascularized tissue that acts as a home for osteoclast and osteoblast progenitor cells, both of which are mediators for bone remodelling (Zhong and Akkus 2011). Bone marrow cells have a mechanosensitive response to mechanical excitation. The bone marrow flows as a fluid medium (0.0072-1.67 ml/min) (Md. Saad et al. 2016; Md Saad et al. 2017)), passing through cancellous bone structure at a body temperature of 37°C. The nutrients supplied to the bones by the bone marrow are affected by the human metabolic system and physiological activities (Syahrom et al. 2014). Deficiency in nutrient supply will affect the ability of cancellous bone to repair and remodel. This will lead to ageing and skeletal diseases. In severe cases bones may even fracture.

A scaffold with a porous structure is known to promote tissues interlocking, cell migration, and nutrient transport as well as osteo-integration with replaced host tissues (Gong et al. 2015). Another advantage of using a porous structure is that the pore size and surface area of the scaffolds can be controlled and regulated precisely to the desired forms, shapes, and structural property (Lewis 2013; Syahrom et al. 2013; Bobe et al. 2013; Cheng et al. 2016). Similar to cancellous bone, different skeletal sites have different porosities depending on their functional properties. The Young's modulus of cancellous bone usually decreases as porosity increases (Shimko et al. 2005). The percentage porosity of human cortical bone is 2%–7% (Renders et al. 2007), while cancellous bone is 70%–95% porous (Snyder et al. 1993; Morgan and Keaveny 2001; Renders et al. 2007).

Commercially available porous scaffolds for orthopaedic applications have been made from non-degradable biomaterials. These porous bone scaffolds are intended to replace the damaged cancellous bone, with the main focus being on hip and knee surgery applications (Balla et al. 2010).

However, compared to Fe-based and newly introduced Zn-alloys, Mg and its alloys are considered to be the most suitable biodegradable metals for potential bone scaffold applications (Staiger et al. 2006; Zheng et al. 2014). Mg can be easily found in human bone tissue since its function is essential in human metabolism (Vormann 2003). Mg holds interesting mechanical properties close to that of human bone (Witte et al. 2008; Gu et al. 2009; Jasmawati et al. 2017). The structural properties of Mg are controllable and can be regulated precisely by constructing a porous structure with a Young's modulus that closely matches cancellous bone (0.01–2.0 GPa) (Gibson 1985; Snyder et al. 1993; Morgan and Keaveny 2001; Renders et al. 2007). The degradation of Mg-based alloys is initiated on the exposed surface area of the materials, which are immersed in a solution of chloride ions in a non-oxidizing medium. These further degrade and extend to the entire surface. The deterioration of materials increases with prolonged time immersion. Usually, assessment of the degradation behavior of porous Mg for bone scaffold applications is determined via static immersion tests, which are also known as the gold standard (Cheng et al. 2016). However, the dynamic immersion test has also been used, transcending the typical techniques, as it integrates the environment of fluid movement passing through cancellous bone (Md. Saad et al. 2016; Md Saad et al. 2017; Md Saad and Syahrom 2018). The degradation rate and structural properties of porous magnesium under a dynamic immersion test with a constant flow has been reported by Md Saad et al. (2016). However, the analysis of morphological changes and growth of new tissues in the biomaterials used for bone scaffold application under such an environment has not yet been reported, and thus could further contribute to the state-of-the-art research in this field.

Micro-CT is a non-destructive technique, which has the proven capability to analyse tissue engineering applications (Hedberg et al. 2005; Mistry et al. 2010; Fischerauer et al. 2013; Yu et al. 2018). The method provides a means to assess the micro-architecture of bone tissues as well as bone scaffolds, before and post-implantation (Hedberg et al. 2005; Mistry et al. 2010; Bobe et al. 2013; Feyerabend 2014). The mechanical integrity of the bone scaffold could be anticipated through the micro-CT technique, in which its morphological changes as a structural support are analysed. The mechanical strength of the bone scaffold is largely determined by its degradation behaviour, especially while the bone is healing (Angrisani et al. 2016).

Hypothetically, large physical changes in porous structures may cause reduced structural properties. Therefore, the correlation between progressive degradation due to the effect of micro-damages and structural properties can be verified through computational works, which also further strengthens results obtained from experimental work. Ideally, the porous scaffold for cancellous bone replacement should function temporarily as a buffer to the damage. In this way, the scaffold could serve to encourage the regeneration of new tissues and act as a structural support while the new tissues grow and the porous scaffold degrades. Still, the degradation time of porous Mg must be monitored to prevent the loss of structural support, and this could be analysed via Finite Element Analysis. To the best of our knowledge, this study is among the first to analyse the morphological changes and structural properties of biodegradable porous Mg for bone scaffold application under a dynamic immersion test using the computational approach.

2.0 Materials and Methods

2.1 Production and degradation of porous Mg

For the purpose of this study, 3 types of cuboid samples (5×5×3 mm) with three different percentages of porosity (30%, 41%, and 55%) are used, as shown in Fig. 1. These cuboids are made from a commercially pure magnesium rod (25.4 mm diameter and 99.9% purity) obtained from Goodfellow Inc, Cambridge, UK, and fabricated using a CNC machine (HAAS, USA). The pore

size of the specimen is 800 μm. Table 1 gives an overview of the geometries of the porous magnesium. After the fabrication process, the specimens were cleansed from any excess materials and chemicals using air jets and an interdental brush (Tepe, USA). Prior to surface polishing, the specimens were immersed in acetone for 15 min. The outer surfaces of the specimens were then ground using #800 and #1200 grit paper and then ultrasonically cleaned in acetone for 15 min. The specimens were desiccated in a vacuum chamber for 1 h prior to degradation tests.

The porous Mg was subjected to a dynamic degradation test under moving simulated body fluid (SBF) at 0.025 m/min with pH and temperature levels of 7.4 and 37°C ± 1°C, respectively (Md. Saad et al. 2016; Md Saad et al. 2017). The dynamic immersion test rig was set to have laminar flow. A peristaltic pump was used to provide a constant flow rate of 0.025 ml/min with a Reynolds number (Re) of 5.44 for the SBF throughout the 2 mm-diameter tube channel. The SBF was made of (8.035g) NaCl, (0.355g) $NaHCO_3$, (0.225g) KCl, (0.231g) $K_2HPO_4.3H_2O$, (0.311g) $MgCl_2.6H_2O$, (39ml) HCl 1.0M, (0.292g) $CaCl_2$, (0.072g) Na_2SO_4, and (6.118g) Tris-buffer. The specimens were immersed for 24 h, 48 h, and 72 h. The tested specimens were then rinsed with deionised water and dried up in a vacuum chamber for 1 h. A diluted chromic acid solution (H_2CrO_4) was used to clean the corrosion products on the tested specimens.

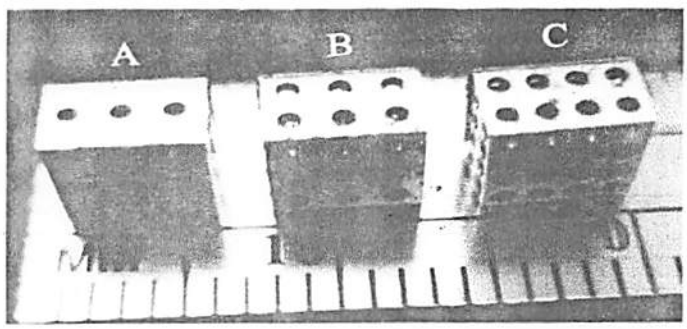


Figure 1The three types of porous Mg specimens used in this study.

Table 1 Overview of the detailed geometries for the porous Mg specimens (Md. Saad et al. 2016; 2017).

Type	Porosity	Surface area	Volume	Mass per Surface area
A	30%	189.30 mm^2	52.87 mm^3	0.44 kg/m^2
B	41%	209.81 mm^2	44.57 mm^3	0.34 kg/m^2
C	55%	225.75 mm^2	33.83 mm^3	0.24 kg/m^2

2.2 Morphological characterisation

Experimentally, the specimens were categorised into 4 groups (prior to and postimmersion test at 0 h, 24 h, 48 h, and 72 h). Each group was replicated three times for each test. Meanwhile, for the computational method, a single specimen from each group was used. The micro-CT images of the cleaned specimens (n = 12) were obtained using a Skyscan 1172 micro-CT device (Kontich, Belgium) at a voxel resolution of 17 μm. The micro-CT images were imported into ImageJ (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA) to analyse the

parameters of ratio of degraded volume and total volume (bv/tv), surface area, and trabecular separation (Tb.Sp).

Trabecular separation (Tb.Sp) is determined through the local thickness of the selected volumetric region (space) in between average surface-to-surface trabecular. The Tb.Sp at a single point in the porous structure is described as the diameter of the greatest sphere that corresponds within the space, which contains the point, as shown in Fig. 2. The reported Tb.Sp mean number is an arithmetic mean of the pointwise Tb.Sp values. The arithmetic mean is calculated using ImageJ using a BoneJ plugin in the trabeculae thickness section. However, in this case, the voxels representing the non-solid structure parts are filled with the greatest spheres. Thus, Tb.Sp is the average thickness of the filled cavities.

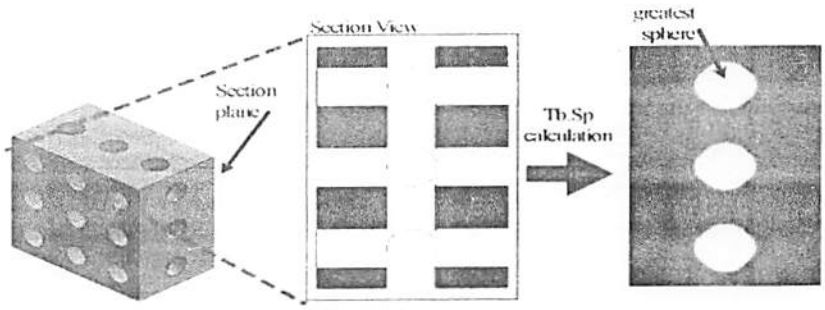


Figure 2 Trabecular separation (Tb.Sp) of Specimen A prior to the immersion test.

2.3 Segmentation Technique

Raw data from the micro-CT images in dicom format were used to reconstruct the threedimensional model of the specimens. The data set comprised 2D images of porous Mg with a slice thickness of 0.172 mm. The images were imported into Mimics software (Materialise, Belgium) to construct a 3D model of the degraded porous Mg, as shown Fig. 3. A region of interest (ROI) was defined and reduced as much as possible, so that the specimen would be encapsulated by the minimum number of black voxels (i.e. air). The ROI is defined by a rectangular shape of 4 mm × 6 mm (larger than the cross-sectional area) and a height of 6 mm.

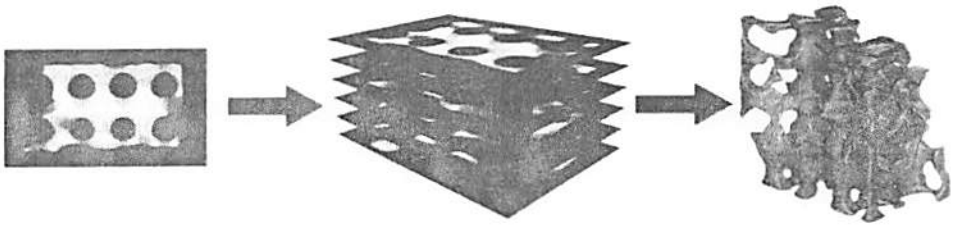


Figure 3 3D reconstruction of Specimen C (percentage porosity of 55%) from micro-CT images using Mimics software after 72 h of immersion time.

The optimal threshold value is defined as that which achieves segmented volumes closest to reality or in other words, when the difference in the volumes, $V_{actual} - V_{model}$, is minimum. The optimal

threshold value can be reached via a manual iterative segmentation in the Mimics Software. The flowchart of the iterative segmentation based on thresholding optimisation is shown in Fig. 4a. The porous magnesium specimen images obtained from the CT scanners consist of grayscale information. Mimics software allows the user to create based on the gray-values (Hounsfield units in CT images) within these images. A gray-value is a number association between the material density of the scanned object and the gray-values assigned to each pixel in the image data. Because of this, Mimics (MIMICS; materialise, Louvain, Belgium) has the flexibility to create models from any geometry distinguishable within the scanned data. By grouping together similar gray-values, the image data can be segmented, and models created. This type of segmentation is called thresholding and yields accurate models. Based on initial testing and trial-and-error we decided to use the Region growing threshold-based (upper/lower) algorithm in our study. First, a range of grayscale values was chosen in order to achieve segmentation of the specimens. Next, the volume model of the specimen was set and a mask computed. The volume of the specimen must be equal to the actual model. The real volume of the scaffold after degradation (V_{actual}) is known from experimental data, for which the actual volume was calculated by dividing the mass of the scaffold by the density of pure Mg (1.74 g/cm^3).

The validation stage compares the volume of the scaffold model and the actual volume. If the condition is not fulfilled, the optimisation stage, which iteratively adjusts the threshold value, is again conducted in order to obtain a model volume that matches the actual volume. This procedure is time consuming because if the grayscale chosen is not appropriate (Fig. 4b), the user will have to start over, change the range of threshold, and compute the mask again. A comparison of the 3D models from the iterative threshold is shown in Fig. 4c.

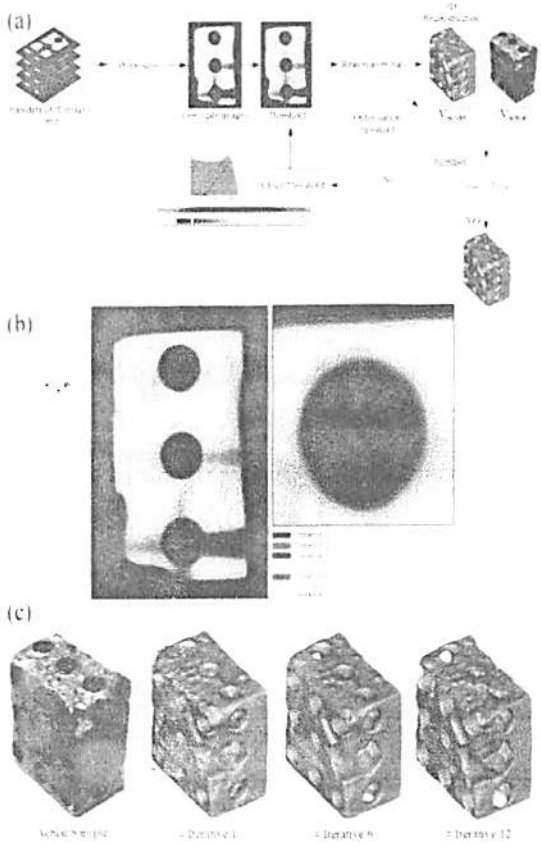


Figure 4 Iterative segmentation: (a) Flowchart of the iterative segmentation based on thresholding optimisation, (b) An example of the scaffold model segmentation based on the iterative

thresholding method in Mimics software, and (c) A comparison of the 3D models from the iterative threshold.

Table 2 shows a comparison of the volume determined from the CT images and the actual volume. From the results, it can be seen that the proposed iterative segmentation method provides reliable results, presenting accurate values that match the intrinsic resolution of the CT images.

Table 2 Summary of the virtual model created with manual iterative threshold segmentation.

Iterative	Threshold Values	Volume of Mimics model (mm3)	Actual volume (mm3)	Percentage Error %
#1	-592	50.58	39.89	26.82
#2	-407	46.32	39.89	16.13
#3	0	41.61	39.89	4.33
#4	110	40.57	39.89	1.72
#5	150	40.19	39.89	0.77
#6	186	39.87	39.89	0.03
#7	200	39.73	39.89	0.39
#8	240	39.41	39.89	1.19
#9	267	39.16	39.89	1.82
#10	300	38.89	39.89	2.49
#11	1076	32.53	39.89	18.53
#12	1416	28.83	39.89	27.72

2.4 Degradation Determination

Degradation rate (DR) was approximated using micro-CT through the reduction in volume (ΔV), instead of using weight loss by means of experiment. The calculated volume loss from the original (prior to testing) volume to the current (after testing) volume at a particular immersion time was then converted to mass loss using the density (ρ) of Mg (1.74 g/cm^3), resulting in the degradation rate (mg/cm^2/d), as per Eqn. (2.1):

$$DR = \frac{\Delta W}{At} \qquad (2.1)$$

Where ΔW is the mass loss, A is the surface area of the bone scaffold, and t is the immersion time.

2.5 Compression test

Compression testing was performed using a universal testing machine (The FastTrack 8874, Instron, Norwood, USA). The mechanical properties of porous Mg specimens before and after immersion tests were evaluated under the compression test, at a strain rate of 0.005/s using a 25 kN load-cell. The elastic modulus was determined as per ASTM D1621 and ISO 13314 standards. The testing procedure for both standards are designed to measure the mechanical properties of rigid cellular plastics under compression loadings (Vesenjak et al. 2016). Three replications were done.

The reconstructed 3D models were converted to finite element meshes. Marc Mentat software (MSC Software, Santa Ana, CA) was used for finite element simulation. The 3D model of the degraded porous Mg was assigned homogenous, isotropic, and elastic-plastic properties. Prior to the numerical analyses, the Young's modulus of pure solid magnesium was determined by conducting compressive tests using solid samples. An average value from the compressive tests was then used as input for all numerical simulations. An ideal plasticity model was assumed for all simulations presented in the present investigation. The Young's modulus value was set to 3.5 GPa and a Poisson's ratio of 0.35 was chosen to correspond to the properties of dense trabecular bone tissue with an apparent density of 1.74 g/cm^3. The model was developed with tetrahedral, pentahedral, and hexahedral elements with an average value of (107403).

The compression test was simulated via Finite Element Method to determine the behavior of the structure of non-degraded and degraded porous Mg. The boundary conditions used in the Finite Element Analysis (FEA) are shown in Fig. 5. A time-dependent displacement boundary condition was defined on the top surface to simulate the moving platen of an experimental test rig. Time-dependent boundary conditions refer to the nodes assigned on the top surface to be solved in a finite domain. In addition, a zero-displacement boundary condition was assigned to the opposite surface in its normal direction, simulating a stationary compression platen. Nodes included in this boundary condition were confined only in the ydirection but were allowed to move freely inside the x-z plane. The macroscopic compression strain limit was set to $\varepsilon = 0.3$ for the computational tests. A plastic strain of 30% was chosen for the scope of this study since plasticity is predicted to occur within the specified plastic strain range.

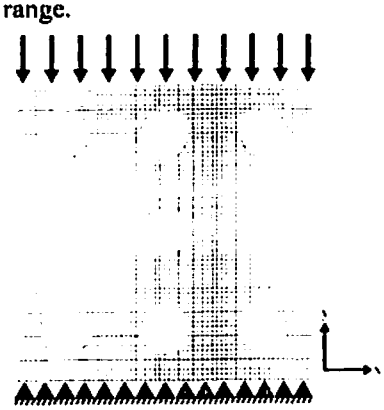


Figure 5 Boundary conditions applied on the porous Mg.

2.6 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corp. USA). Pearson's correlation test was conducted to predict the structural properties of porous Mg by volume fraction (bv/tv), bone surface area, and trabecular separation (Tb.Sp).

3.0 Results

3.1 Degradation Characterisation

Figure 6 shows the charts of degradation of the porous Mg specimen determined using micro-computed tomography measurement. Figure 6a shows the relative volume loss of porous Mg with increased percentage difference in porosity as the time of immersion increased. The loss of volume in the specimen increased as the percentage of porosity increased. Figure 6b demonstrates the relative surface area loss of the specimen, which increased as the time of immersion increased. The specimen with a higher percentage of porosity shows a huge loss of surface area compared to others. The loss of surface area curve for Specimens A and B shows a similar trend, in which the increment in surface area loss gradually decreases as time of immersion increased. Meanwhile, Specimen C was observed to lose more surface area as immersion time increased. Figure 6c shows the degradation rate of the porous Mg specimen with different percentages of porosity using original surface area. After 24 hours of immersion time, Specimen B showed a higher degradation rate followed by Specimens C and A. Meanwhile, after 48 hours, all the specimens showed almost similar degradation rates. After 72 hours, Specimen C showed the highest degradation rate followed by Specimens B and A. Figure 6d shows the degradation rate of specimens with different percentages of porosity, which were calculated using surface area and determined by micro-CT measurement at their respective immersion time. The degradation rate of Specimen C was higher after a 24-hour immersion time, followed by Specimens B and A, which showed a similar trend after 48 and 72 hours. Meanwhile, after 72 hours, the degradation rate of Specimen C increased compared to the other specimens, which saw a decrease.

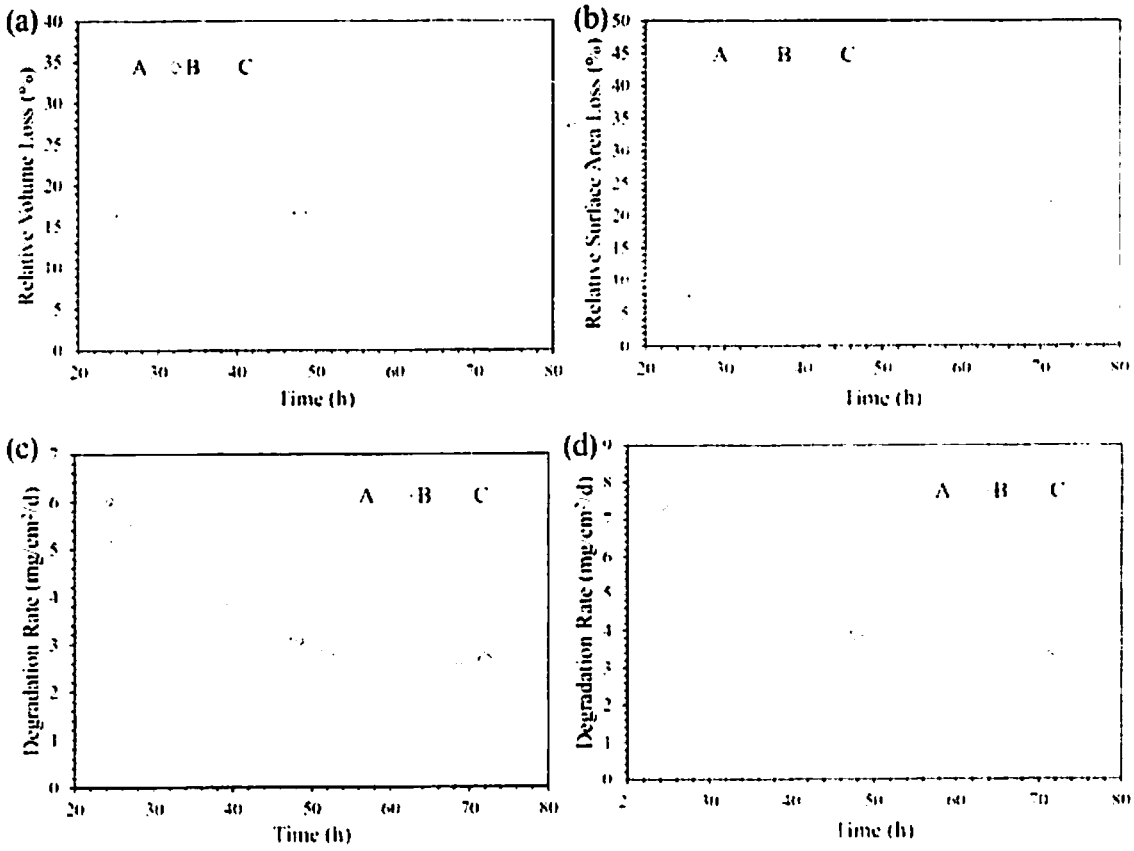


Figure 6 Degradation characterisation: (a) relative volume loss (%), (b) relative surface area loss (%), (c) degradation rate of specimens using original surface area, and (d) degradation rate of specimens using degraded surface area.

3.2 Mechanical Characterisation

Figure 7a shows the elastic modulus of the specimens determined by means of experiment and simulation. The elastic modulus determined by simulation is in good agreement with the experiment, whereby all samples show a clear decreasing trend in modulus value with increasing percentage of porosity and immersion time. Figure 7b shows a comparison of compressive stress-strain curves between the experimental and finite element simulations for Specimen A prior to the immersion test (A0). When the strain exceeds 0.05, the experimental data dropped significantly and vice versa for the simulation.

Figure 8 demonstrates the principal elastic strain of the specimens with different percentages of porosity after the dynamic immersion test under a uniaxial compression test. Specimen A shows an oblique fracture at 45 degrees, while Specimens B and C experienced global fracture. From Fig. 8, the experimental results and predictions from FEA are also compared within the same group of specimen morphologies. It can be concluded that elastic strain from the FEA results suggest a similar collapsing pattern indicated by red dashed lines. It is interesting to note that some simulation results are slightly above the range of experimental data. This may be explained by the fact that numerical models are selected from one of three specimens used for the experimental tests.

In the case of B24, for example, our result from the numerical simulations over-predicted the elastic modulus for this group sample's morphology.

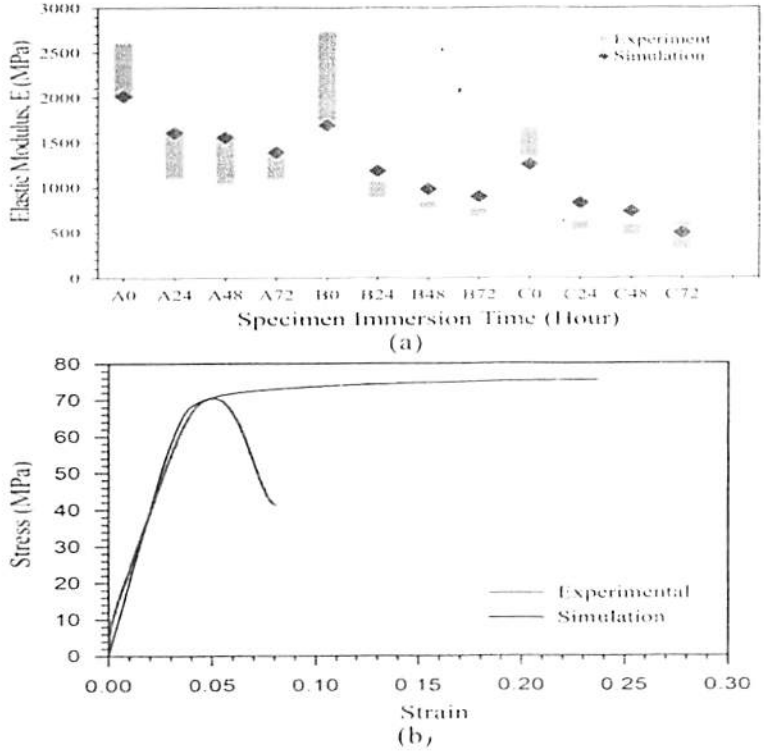


Figure 7 (a) Elastic modulus of the specimens determined by means of experiment and simulation (Md. Saad et al. 2016) and (b) Comparison of compressive stress-strain curves between the experimental and finite element simulations for Specimen A prior to the immersion test (A0).

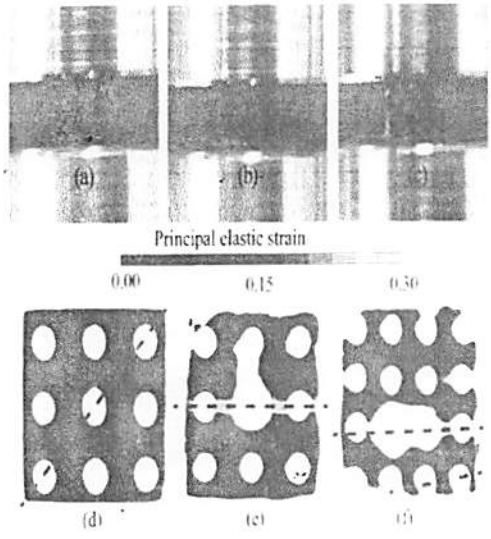


Figure 8 Contour plots of the fracture patterns after the dynamic immersion test: (a) Specimen A, (b) Specimen B, and (c) Specimen C, and principal plastic strain contours from FEA: (d) Specimen A, (e) Specimen B, and (f) Specimen C.

3.3 Morphological Characterisation

Figure 9 shows the charts of morphological changes of porous Mg after being subjected to the dynamic immersion test for up to 72-hours. Figure 9a shows the volume fraction changes in the specimens. The volume fraction of each specimen is seen to decrease over time. After a 72-hour immersion time, the percentage of porosity (1 − volume fraction) for Specimens A (30%), B (41%), and C (55%) increased to up to 50%, 53%, and 72%, respectively. Figure 9b illustrates the changes in surface area of porous Mg for each specimen, which decreases as immersion time increases. The surface area of Specimens A and B gradually decreased by up to 17% and 22%, while Specimen C saw an abrupt decrease in surface area by 44%, after the 72-hour immersion time. Figure 9c shows the changes in trend for trabecular separation in each specimen, which increased as immersion time increased. After the 72-hour immersion time, the trabecular separation changes for Specimens A, B, and C were 47%, 46%, and 69%, respectively.

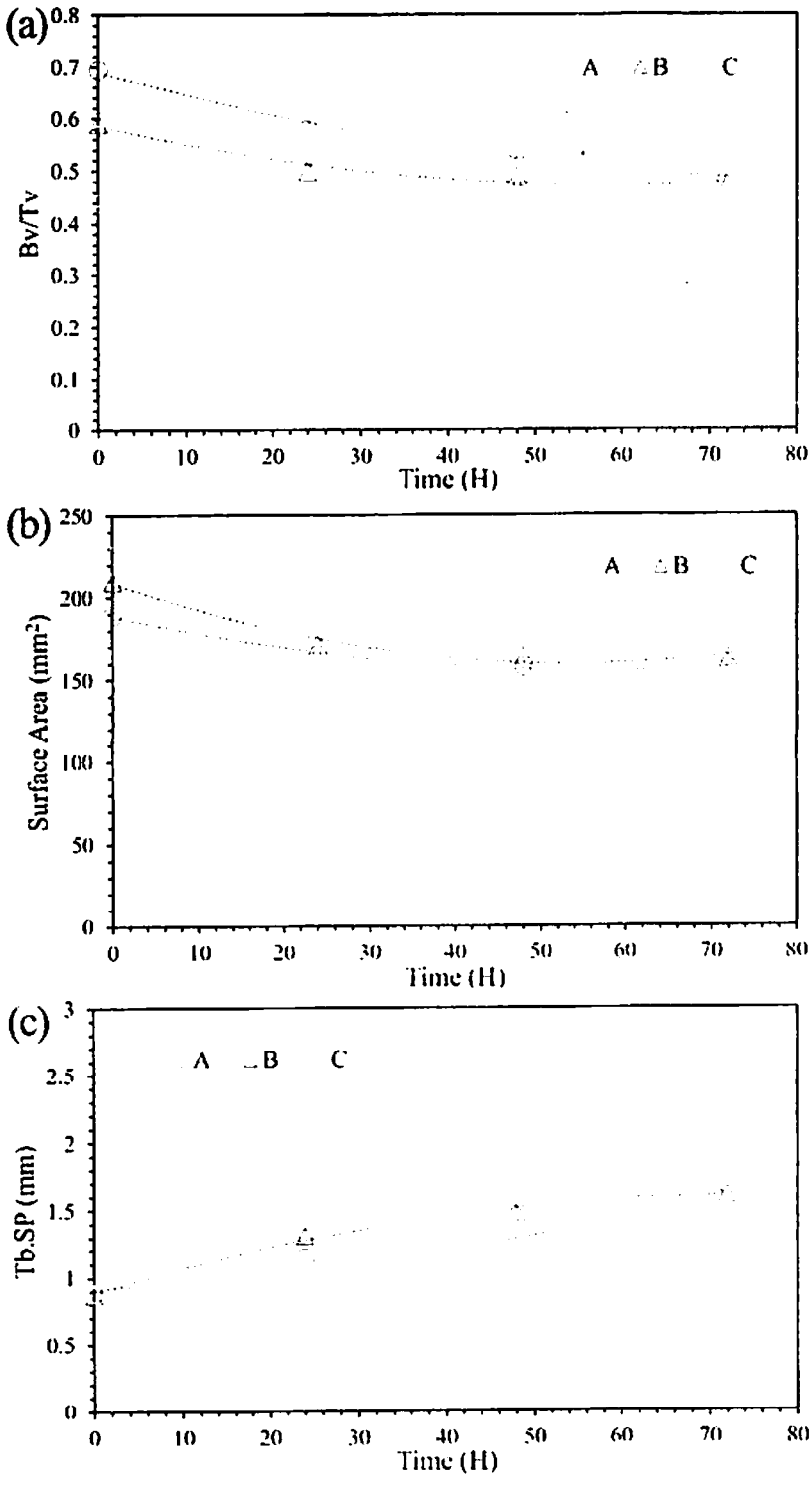
3.4 Mechanical Degradation

Figure 10 shows the correlation between elastic modulus and morphological parameters of porous Mg before and after being subjected to tests. The elastic modulus from the simulation was used to analyse for any correlation with the morphology of the specimens. The morphological parameters acquired from the micro-CT analyses are volume fraction (Bv/Tv), surface area, and trabecular separation (Tb.Sp).

Figure 10a shows the correlation between the elastic modulus and volume fraction (1 – porosity) of the specimens. The specimens with a higher volume fraction demonstrated a higher elastic modulus. As the time of immersion increased, the volume fraction as well as the elastic modulus for all specimens decreased in an orderly manner. The volume fraction of Specimens B and C showed a sudden drop based on the steep slopes as compared to Specimen A, which gradually decreased. The volume fraction of Specimen C was greatly reduced after 72 hours, thus resulting in a low elastic modulus. From the statistical analyses, volume fraction significantly impacted elastic modulus with a p-value <0.005 and the correlation coefficient, r, between volume fraction and elastic modulus was 0.94.

Figure 10b shows the correlation of elastic modulus and surface area of the specimen. The surface area for all specimens decreased in an orderly manner as time of immersion increased, which resulted in decreasing elastic modulus, respectively. Specimens A and B showed almost a similar decline pattern compared to Specimen C. The gradual decrease in surface area after 24-hour immersion time resulted in the smallest changes in elastic modulus as compared to specimens that demonstrated a huge loss in surface area. From the statistical analyses, surface area significantly impacted elastic modulus with a p-value <0.005 and the correlation coefficient between surface area and elastic modulus was 0.57.

Figure 10c shows the correlation between elastic modulus and trabecular separation of the specimen. The trabecular separation for all specimens increased in an orderly manner, which caused elastic modulus to decrease accordingly. The initial value of trabecular separation for all specimens is almost similar; however, the result is different for elastic modulus. The large changes in trabecular separation for Specimen C correlated to a huge drop in elastic modulus after the 72-hour immersion time. From the statistical analyses, trabecular separation significantly impacted elastic modulus with a p-value <0.005 and the correlation coefficient between trabecular separation and elastic modulus was -0.85.



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390 Figure 9 Morphological changes in porous Mg under dynamic degradation test: (a) volume 391 fraction (Bv/Tv), (b) surface area, and (c) trabecular separation (Tb.Sp).

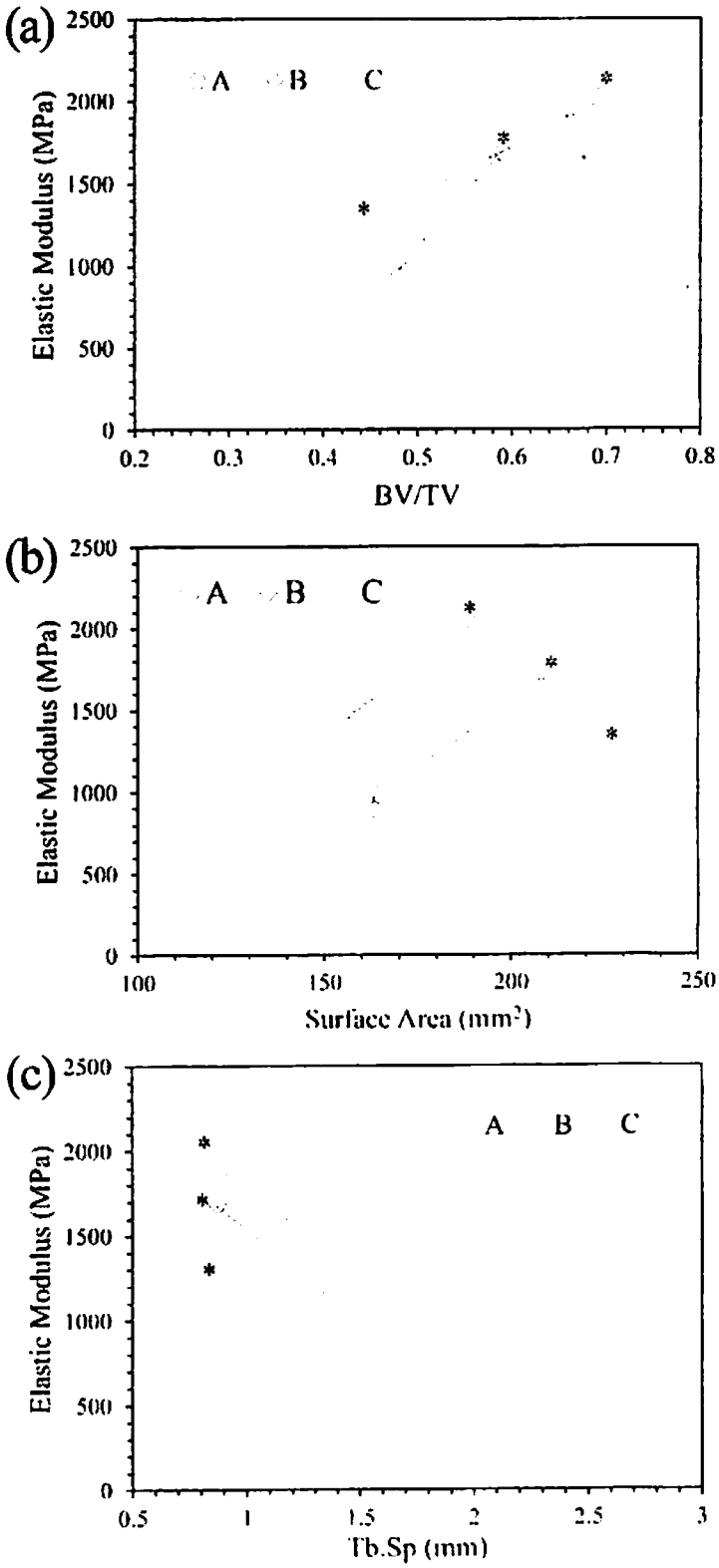

Figure 10 Correlation between elastic modulus and morphological parameters: (a) volume fraction (Bv/Tv), (b) surface area, and (c) trabecular separation (Tb.Sp). (Note: * denotes the specimen before being subjected to the test)

4.0 Discussion

It has been suggested that porous Mg be used as cancellous bone replacement due to its mechanical properties, which closely match cancellous bone properties. The nature of the cancellous bone environment encourages the bone remodelling process to happen with the condition that physiological activity be performed. This activity induces pressure differentials on the bone, thus causing the bone marrow to flow. The movement of the bone marrow passing through the porous structure triggers mechanical stress through mechanobiological signalling that activates the bone remodelling process. Thus, these conditions preserve bone quality. Therefore, once porous Mg is implanted, it will be exposed to the cancellous bone environment, and will not only serve as a device to enhance tissues growth, but also most importantly to act as a load bearing structure during the bone healing process. The dynamic degradation of porous Mg under a simulated human cancellous bone environment has been reported elsewhere (Md. Saad et al. 2016). However, there are some parameters such as morphological parameters that cannot be fully obtained through experimental works. Therefore, micro-CT measurement was used to acquire the comprehensive mechanical characteristics of porous Mg as a load bearing structure. Degradation rate by means of micro-CT determination is a useful non-destructive measurement technique especially for clinical usage. Besides being advantageous for clinical use, this technique also enables the acquisition of morphological parameters, which might be impossible to obtain through experimental works. Additionally, the rate of new tissue matrix growth can also be identified using this technique. Hence, the impact of morphological indices such as volume fraction (bv/tv), bone surface area, trabecular thickness (tb.th), and trabecular separation (Tb.Sp) on the elastic modulus of porous Mg can be determined. However, this method is costly.

Relative volume loss determined by means of micro-CT analysis, as shown in Fig. 6a, demonstrate that relative volume loss increases as the time of immersion increases, whereby the specimen with a higher percentage of porosity showed greater volume loss compared to others. The pattern of volume loss is in agreement with relative mass loss reported elsewhere (Md. Saad et al. 2016). Meanwhile, through the micro-CT analysis, the loss of surface area could also be measured after the specimen was subjected to the dynamic degradation test, which might have been impossible to obtain through experimental works (Feyerabend 2014). As presented in Fig. 6b, the relative surface area loss increased as the time of immersion increased; the percentage of porosity also increased. The larger the exposed surface area of Mg to the fluid medium, the faster the degradation process in proportion to the surface contact available (Bobe et al. 2013). Therefore, the distinct trend in surface area loss for Specimen C after the 72-hour immersion time was identified due to its larger surface contact resulting in more degradation than the others. Figures 6c and 6d show the degradation rate of porous Mg determined by means of micro-CT; however, the difference between them is the surface area used, which are the original and degraded surface areas, respectively. Both degradation rate trends demonstrate agreement with previous work (Md. Saad et al. 2016). The degradation rate using original surface area obtained in this study (Fig. 6c) shows a slightly different pattern compared with previously reported work using weight loss measurement. After the 24-hour immersion time, Specimen B degraded faster compared to Specimen C and all specimens showed similar degradation rates after the 48-hour immersion time. In contrast, degradation rates using exact (degraded) surface area at a particular time (Fig. 6d) demonstrated on average a 20% higher degradation rate compared to that of using original surface area (Fig.4c).

Therefore, predictions that take into account the use of actual surface area will result in higher degradation rates. In terms of accuracy, the degradation rate calculation must include the actual value of surface area, so that the real *in situ* degradation rate can be obtained (Bobe et al. 2013).

This present work used twelve specimens and measured their morphological parameters using micro-CT scan, and mechanically characterised them using FEA. The obtained compression test results were then validated with experimental works (Md. Saad et al. 2016). Fig. 7a demonstrates the range of experimental value and the point of simulation results for the compression test. The elastic modulus from the experimental work was in agreement with that of the simulation. This shows that the prediction of structural properties by means of FEA could be used to predict the correlations between the structural properties and morphological parameters of degraded porous Mg. The simulation data shows an underestimated Elastic Modulus at t = 0, and closely overestimated Elastic Modulus from experimental data at t = 24, 48, 72, which is due to the segmentation of the degradation model. Although the percentage error is close to the real structure, we anticipate that the complexity of the degradation model was altered, where a specific region, especially the thin strut of the degraded region showed an insert out element error (overlapping). Meanwhile, Fig. 7b shows good agreement between experimental and simulation data for up to 0.05 strain. However, beyond that, the theoretical stress–strain curve significantly dropped due to plastification and fracture (break) from the experimental work because porous Mg is a brittle material. The simulation curve gradually increased (known as the plateau region) past the 0.05 strain. This is because the FE software only considered up to the yield strength of the material acquired from experimental data. Therefore, the fracture was not taken into account and shows that the stress increased up to 0.3 strain.

The fracture line of porous Mg after the dynamic immersion test, as shown in Fig. 8, indicates that there are two types of fracture: oblique and global. Oblique fracture can be observed in Specimen A from both the experimental and FEA analyses of the magnesium scaffold structures. This type of fracture mode may be attributed to the ductility of the material. Plastification continues to form a band of collapse as it moves to randomly distributed weak regions. For Specimen A, an oblique fracture was observed in the experimental tests and FEA simulation. A collapse band of 45° can be seen from both Figures 6a and 6d. In addition, a study reported that most cancellous bone generally have global fractures, however, there are still some cancellous bone specimens that undergo oblique and localized fracture (Syahrom et al. 2011). The random alteration of porous Mg due to degradation has caused large changes to the specimen with a higher percentage of porosity. The localised degradation has weakened the struts of the specimen, which was identified to cause Specimen C (Fig. 8c) to show a two-line fracture. These fractures are associated with high trabecular separation, as shown in Fig. 9c (Perilli et al. 2008). This information is crucial in applying the bone scaffold for load bearing purpose. The fracture characteristic is similar to actual cancellous bone, which could represent the damage behaviour of the scaffold once implanted to prevent different directional stress effects.

The alteration to the structure due to degradation mechanism has caused significant changes to the morphological indices of porous Mg as immersion time increased, as shown in Fig. 9. These changes are important for predicting the reduction in structural properties of porous Mg as a bone scaffold that serves as a load bearing structure while the bone heals and new tissues form. The volume fraction, surface area, and trabecular separation of the specimen were significantly correlated over immersion time. The specimen with a higher percentage of porosity was vulnerable to greater morphological change due to dynamic degradation mainly because of the larger surface area contact. However, it is not typical for a porous specimen with a higher percentage of porosity to directly correlate with a high surface area as well. As reported by Syahrom et al. (Syahrom et al. 2013), a porous specimen usually has a similar percentage of porosity with different surface areas due to the design of its morphological structure.

The effects of morphological changes on the elastic modulus are shown in Fig. 10. All morphological parameters were reduced significantly after the 24-hour immersion time, except for Specimen C, which showed gradual reduction after 72 hours. This is related to the rigorous degradation process

that occurs in the first 24-hour immersion time. This results in shrinkages to the structure and reduces the effective modulus. As the time prolongs, the passive layer built up on top of the surface slows down the degradation rate, which causes little change to the structure (Md Saad et al. 2017). The morphological parameters such as volume fraction, surface area, and trabecular separation had a strong correlation with elastic modulus, as demonstrated in Figs. 10a, 10b, and 10d (Syahrom et al. 2011).

It should be noted that several assumptions were made in this study. The work was also subject to some limitations. One limitation is that the micro-CT measurement of each specimen was not repeated. Currently, the study managed to obtain the micro-CT data sets of twelve specimens. The selection of the specimen from one group (for example: after the 24-hour immersion time group) was taken randomly, for which the assumption is their degradation is almost similar based on macro-level sight. This is where computer simulation is preferred because it does not cost as much as micro-CT measurements.

Conclusion

The present work evaluated the connection between morphological changes and structural properties of porous Mg under a dynamic immersion test. A micro-CT technique was used to quantitatively measure the degradation rate and morphological changes of porous Mg. From the data scan, 3D models were reconstructed and their structural properties analysed using FEA. The correlation between the acquired structural properties with morphological parameters was identified and their level of significance investigated.

1. The percentage of volume loss from computational works is in agreement with relative mass loss done experimentally.
2. The degradation rates determined using exact (degraded) surface area at particular immersion times (Fig. 6d) were on average 20% higher compared to degradation rate obtained using the original surface area.
3. Dynamic degradation is shown to significantly impact the morphological changes of porous Mg such as volume fraction, surface area, and trabecular separation with pvalues <0.005. These in turn affect the structural properties of porous Mg.

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Conflict of Interest

The authors declare no conflict of interest.

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Lampiran 2
Acceptance letter

Fwd: Editor's decision on ABME-D-17-00524
Dari:DR. ARDI (ardi@utm.my)
Kepada:amirputra@utm.my; mayub@utm.my; dianagustin_fkg@yahoo.co.id; dian-a-w@fkg.unair.ac.id; hasanbas1960@gmail.com; akbar.teguh.prakoso@student.unsri.ac.id
Tanggal:Selasa, 29 Agustus 2017 10.02 WIB

Alhamdulillah, our paper accepted.
Congratulations to all.

Ardi
---------- Forwarded message ----------
From: "Annals of Biomedical Engineering (ABME)" <em@editorialmanager.com>
Date: 29 Aug 2017 1:31 am
Subject: Editor's decision on ABME-D-17-00524
To: "Ardiyansyah Syahrom" <ardiyans@gmail.com>
Cc:

CC: jdstitzel@gmail.com, jstitzel@wakehealth.edu

Dear Dr. Syahrom,

Your manuscript, "The impact of dynamic degradation on the morphological and mechanical characterization of porous Magnesium," by Dr. Ardiyansyah Syahrom (Corresponding Author), Amir Putra Md Saad, PhD; Akbar Teguh Prakoso; Mohd Ayub Sulong, PhD; Hasan Basri; Dian Agustin Wahjuningrum, has been reviewed by an Associate Editor and Referees who specialize in the subject matter addressed by the submitted material. The general and specific comments of each referee are included with this email (at bottom). The referees feel the study addresses an important issue and agree that the results are interesting, but feel that a number of significant revisions need to be made before we can further consider your manuscript. Our decision is: Major Revision.

If you decide to revise the manuscript and resubmit it, please include a point-by-point response to the referees' comments. Please also denote changes in the text of your revision by using an alternate color font or highlighting.

Submit the revision at the journal's website.

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Please resubmit your manuscript on or before 27 Sep 2017.

If you decide not to revise, we would appreciate if you let us know by clicking the "Decline to Revise" link.

Please take care to follow all the instructions. Thank you very much.

Sincerely,

Joel Stitzel, Associate Editor and Scott I Simon, Deputy Editor-in-Chief
Annals of Biomedical Engineering

COMMENTS FOR THE AUTHOR:

Additional reviewers were added due to a large number of declined invitations to reviewers early on. The majority of reviewers recommend Major Revision. Please be aware that the author(s) must address issues sufficient to result in 'minor revisions' or 'accept' for the next round, as another major revision round will not be permitted. Please use this information and the reviews provided to make a decision about resubmitting a revised manuscript.

Reviewer #1: The manuscript was well written and the results were clearly presented. The research topic is appropriate to the general readership of the Journal.

Several major concerns of the study have been noticed.
(1) In the Introduction it was not clear why the authors would want to use FEA in the current study. The authors did compression tests on the samples which provided properties of the sample, so I do not see the point of FEA here. If this is addressable, earlier FEA work in the literature, that investigates similar material/structure and is done either by the authors or by others, should be reviewed and included in the Intro. Also, what was the hypothesis of the study? This should be included as well, maybe in the end of the Intro.

(2) Compression tests were done to the three types of samples. Since the same material was used to make the 3 samples, their mechanical properties should be the same. What measured by the authors from the compression tests should be structural properties, but not mechanical properties, due to various locations of the holes in the material. Please clarify.

(3) In Methods, during compression tests, "the elastic modulus was determine as per ASTM D1621 and ISO 844 standards", this determination should be included in details, at least the standards should be briefly described. Also, why consider these properties as mechanical properties, rather than structural properties, as mentioned above in an earlier comment.

In addition, a couple minor concerns are listed as well.
(1) Figure 6 showed the fracture patterns (principal elastic strain) during the compression tests. Since FEA was used to simulate these tests, can the authors compare and show the principal strain profiles from FEA versus these contour plots?

(2) It is suggested that the authors include a paragraph in Discussion to talk about the limitations of the current study.

Reviewer #2: In this work, authors use cuboid samples of Mg with varying porosities and undertake a immersion test under moving simulated body fluid. Micro CT and FEM analysis is done on the samples to predict elastic responses which are found to be close to those observed experimentally. All of the methods presented for experiments and FEM as well as micro CT are fairly standard and the reviewer does not see any new novelty or science contribution of this work. The changes or small differences observed between experiments and models are also not evaluated in any detail. It is not clear to the reviewer what the specific contribution of this work is and how it furthers current state of the art in scaffold biomaterials.

Reviewer #3: This manuscript is a continuation of a previously published work [1] investigating morphological and mechanical properties of porous magnesium cubes (5 x 5 x 3 mm) after degradation via dynamic immersion. The specimens presented in this manuscript are the same specimens presented in two previous works [1,2]. The distinction between this manuscript and [1] is that this work evaluates degradation using CT-image volume instead of by mass, incorporates a finite element model, and evaluates additional morphological parameters (volume loss, surface area, and trabecular separation). There several major issues that dampen enthusiasm for this manuscript:

Major Issues

1. The purpose and use of the finite element model is unclear. As presented, it appears an FE model was created merely because it could be created, but without any goal in mind. The model does not add information to the manuscript over that of the physical compression test data (originally presented in [1]). Further, the development, details and justification for parameters in the model are poor. Specifically, the material properties of bone are used to represent a magnesium material without justification. Appropriate material parameters for an elastic-plastic material model are not provided, and it is unclear that an elastic-plastic model is actually being used. A compression strain limit of 0.3 was used for the FE model without justification. There are no details of the results from the model other than an 'elastic modulus' from simulations of each degraded specimen. There is no description of how this 'elastic modulus' was extracted from the simulations. There is no comparison of how the stress-strain curves from the simulation compare to the stress-strain curves (which are non-linear) from the experimental data [1].
2. Only a single specimen of each porosity at each degradation time was characterized. This study does not capture the experimental variability of porous magnesium degradation.
3. There is no upfront indication that the work presented in this manuscript (specifically, the dynamic immersion tests, CT imaging, compression tests, and one method for calculating degradation rate) were previously performed and already published by the same authors [1]. No new experiments have been performed. It is not until the results and discussion that there appear to some clarification of what data is already published and what data is new.
4. It is unclear what the objective of the manuscript is. It is unclear from the introduction how it builds, differs from, and adds to the previously published work.

Additional issues are as follows:

1. Section 2.1. "A commercially pure magnesium rod (25.4 mm in diameter and 99.89% purity, as shown in Fig. 1, was used." It is unclear how this statement relates to Figure 1. This sentence also appears incomplete. I imagine the cubes were made from the rod, but this is not explicitly stated.
2. Section 2.1. A brief description of the flow device is needed to understand the experiment.
3. Section 2.1. Which body fluids does the SBF simulate? What is it made of?
4. Section 2.2. It is unclear why the volume of the cube is called 'bone volume' when it is not made of bone, but magnesium. Since the context is to evaluation degradation in volume. A term such as degraded volume would be more descriptive to the measure.
5. Section 2.2. There is no description of how trabecular separation was calculated or determined.
6. Section 2.3. The text says that tetrahedral elements were used, but Figure 3 shows a hybrid mesh. Please clarify. Are there 3D images of the mesh? Was a convergence study performed? What are the elements and quality of the elements for each model? What do the simulation results look like?
7. In section 2.5, it states that multiple linear regression analyses were performed, but results indicate correlation analyses were performed. Please clarify. Correlation coefficients should be provided in addition to the p-value. If multiple linear regression was indeed performed, what were the variables in your multiple regression equation? Were interaction effects investigated? The results seem to indicate that a single linear regression may have been performed for each variable rather than a multiple regression (which combines all variables into a regression equation).
8. Section 3.4. Line 31. Strut thickness was not mentioned in the methods and no data is presented anywhere for it in the manuscript.
9. Section 3.4. What quantitative metric was used to distinguish a sharp change in slope versus a gradual change in slope.
10. Section 3.4. When performing the correlations, were all specimen data points combined? Or were analyses for A, B, and C performed separately?
11. Section 4.0. "Oblique fracture was observed only in specimen A due to the high stiffness of the specimen compared to the others." Oblique fractures in solid structures are not a function of the stiffness of the material, but rather of the ductility or brittleness of the material. The referenced citation does not evaluate fracture, so it is unclear how this statement is supported by the data in the present manuscript or from the provided reference.
12. Section 4.0 "This is related to the rigorous degradation process within the first 24 hours of immersion time, which causes shrinkages to the structure and reduced elastic modulus." Is this sentence referring to the 'effective' modulus? Or was there a proposed reduction in the mechanical properties of the magnesium? Clarify.
13. Figure 2. Which specimen is shown? Porosity? Immersion time?
14. Figure 4. Why are non-linear fits applied to the data? Was a non-linear regression analysis performed?
15. Figure 5. It is unclear how there is an experimental range for elastic modulus when only a single specimen was tested for each porosity and immersion time. Further, from [1] it appears that the data is non-linear. How was elastic modulus extracted?
16. Figure 5. The experimental data is from [1] or [2] and should be referenced in the caption appropriately.
17. Figure 6. The caption does not describe the image. There is no contour plot of principal elastic strain in the image.
18. Figure 7. Why were non-linear fits used for (a) and (b) and a linear fit used for (c)?

Clarify.
19. Figure 8. There is a note in the caption that does not seem to be related or used in the figure.

[1] Md Saad, A, et al. 2016. Dynamic degradation of porous magnesium under a simulated environment of human cancellous bone. Corrosion Science. 112: 495-506
[2] Md Saad, A, et al. 2017. The influence of flow rates on the dynamic degradation behavior of porous magnesium under a simulated environment of human cancellous bone. Materials & Design. 122: 268-279

Reviewer #4: Review of the paper entitled:
"The impact of dynamic degradation on the morphological and mechanical characterization of porous magnesium"
This paper addresses experimental and computation study of a dynamic degradation of porous magnesium. The subject is of clinical interest. The authors provide strong motivation for addressing this problem.
The reviewer has several reservations/questions about this paper.
1. The paper lacks sufficient detail on the problem studied. What type of porosity is introduced? Table 1 gives information about the surface area, volume, and the mass per surface area. But what is the real architecture? Photograph in Figure 1 has more detail. What are the dimensions of the holes? It looks like circular holes are bored with a CNC machine in two orthogonal directions. What do the authors mean by "trabecular separation"? Information on the diameter, distance between them and distances from the edge are important for reproducibility of results.
2. Micro-computed tomography (micro-CT) was used to image samples before and after degradation. How did the porosity measured using the micro-CT images of the intact samples compare with the actual porosity which could be estimated from dimensions of the samples and sizes of the holes?
3. What type of threshold method was used to make sure that surfaces are captured?
4. What was the nature of degradation? Was it uniform or non-uniform? The third image in Figure 2 shows a highly non-uniform porosity. Why?
5. How many samples were used for each geometry?
6. How was the elastic modulus computed using the finite element simulations? Please provide details about the method. Also, please clearly state boundary conditions, in addition to showing it on the Figure 3.
7. In section 2.5 it is stated that statistical analysis was performed. What was stochastic in this problem? How many samples were used for each case?
8. It is not clear what is visualized in the Figure 6? Where are strain contours?
9. Figure 5 shows that finite element model is sometimes above and sometimes below the experimental data. Why?
10. What is the significance of this study? What was learned? How can these results can contribute to knowledge? Please strengthen the conclusions. How robust are these results? Would alternate porosity architectures give similar conclusions?
In summary, this paper requires a careful revision where more details are provided on the conducted experiments and simulations. Also, more clear explanation should be provided on how these results can be used. Finally, as paragraph on what are the limitations and possible extensions of this study would be useful.
Thus, this paper is recommended for publication in the Journal of Annals of Biomedical

Engineering but only after a major revision.

Reviewer #5: General comments
The authors presented an interesting study entitled "The impact of dynamic degradation on the morphological and mechanical characterization of porous Magnesium". The manuscript addressed one of the important issues related to bone substitutes: mechanical characterization after the material degradation by biological fluids. It is possible to be acceptable to publication after a revision. In general, the study is recommended to publication.
Here are some points and suggestions:

Title
It would be interesting to add the term "scaffold" in the title "...of porous magnesium scaffold"

Introduction
The introduction presents important informations about the material and its applications. But the last paragraph is confused:
- "Micro computed tomography (micro-CT) is known as a non-destructive technique...This technique requires the morphological parameters of the material, which is almost impossible to obtain via experimental works. However, finite element analysis (FEA) can be used to measure the morphological changes...". What is the non-destructive technique, the micro-CT or FEA? And to measure the morphological changes, it would be the micro-CT instead FEA?

Materials and Methods
The authors described the material characteristics and the methods. Some points are not clear for the readers:
- According cited in the abstract, the N of the sample is 12? How many per group? Please, these points must be written clearly. The three levels of porosity of the samples were chosen according the bone types? If yes, it could be described.
- About the degradation simulation the authors cited the "simulated body fluid". Is it a commercial material. The authors could describe how this fluid was obtained.
- The authors used an elastic modulus E=3.5 GPa. But they extracted the means from the experimental test. It should be interesting to use the elastic modulus from experimental analysis in FEA.
- Although the authors did a compression test. Suggestion: But it could be interesting to perform other tests as tensile, shear or bending to simulate the different mechanical stimulation in human skeletal structure.
- The authors decribed a finite element mesh as a tethraedral mesh. But the figure 3 shows a mixed mesh, with hexaedric and tethraedric elements.

Results
- The text style was written as figure legends. This style becomes confused for reading. It is recommeded to revise and rewrite as a description. Suggestion: For the figures, it would be interesting a figure of FEA result, showing the 3D models after loading.

Discussion
- The discussion was well described.

Conclusion
- The conclusion was well described.

Lampiran 3
Certificate Poster Presentation

International Federation of Endodontic Associations

The 11th IFEA World Endodontic Congress 2018 Seoul

KAE

Certificate of Presenter

We herewith certify that

Dian Agustin Wahjuningrum

has participated as a Poster Presenter in the IFEA 11th World Endodontic Congress held from October 4 to 7, 2018 in Seoul, Korea.

Mark F Wotzke
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Hyeon-Cheol Kim
Director
Scientific Committee, The IFEA 11th WEC 2018 Seoul

Lampiran 4
Draft Paten Sederhana

KEMENTERIAN HUKUM DAN HAK ASASI MANUSIA R.I.
DIREKTORAT JENDERAL KEKAYAAN INTELEKTUAL

Dibuat rangkap 2

Formulir Permohonan Paten

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Tanggal pengajuan	:
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Dengan ini saya/kami [1]:

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Mekanisme Optimasi Scaffold Magnesium menggunakan Stem Cell

Permohonan paten ini merupakan pecahan/ Perubahan dari permohonan paten nomor : -	[ ]
Diisi oleh petugas (72) Nama dan kewarganegaraan para inventor : - DIAN AGUSTIN WAHJUNINGRUM warga negara INDONESIA - LATIEF MOODUTO warga negara INDONESIA	[ ]
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Saya/kami usulkan, gambar nomor 2 dapat menyertai abstrak pada saat dilakukan pengumuman atas permohonan paten (UU No. 13 Tahun 2016)	[ ]
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